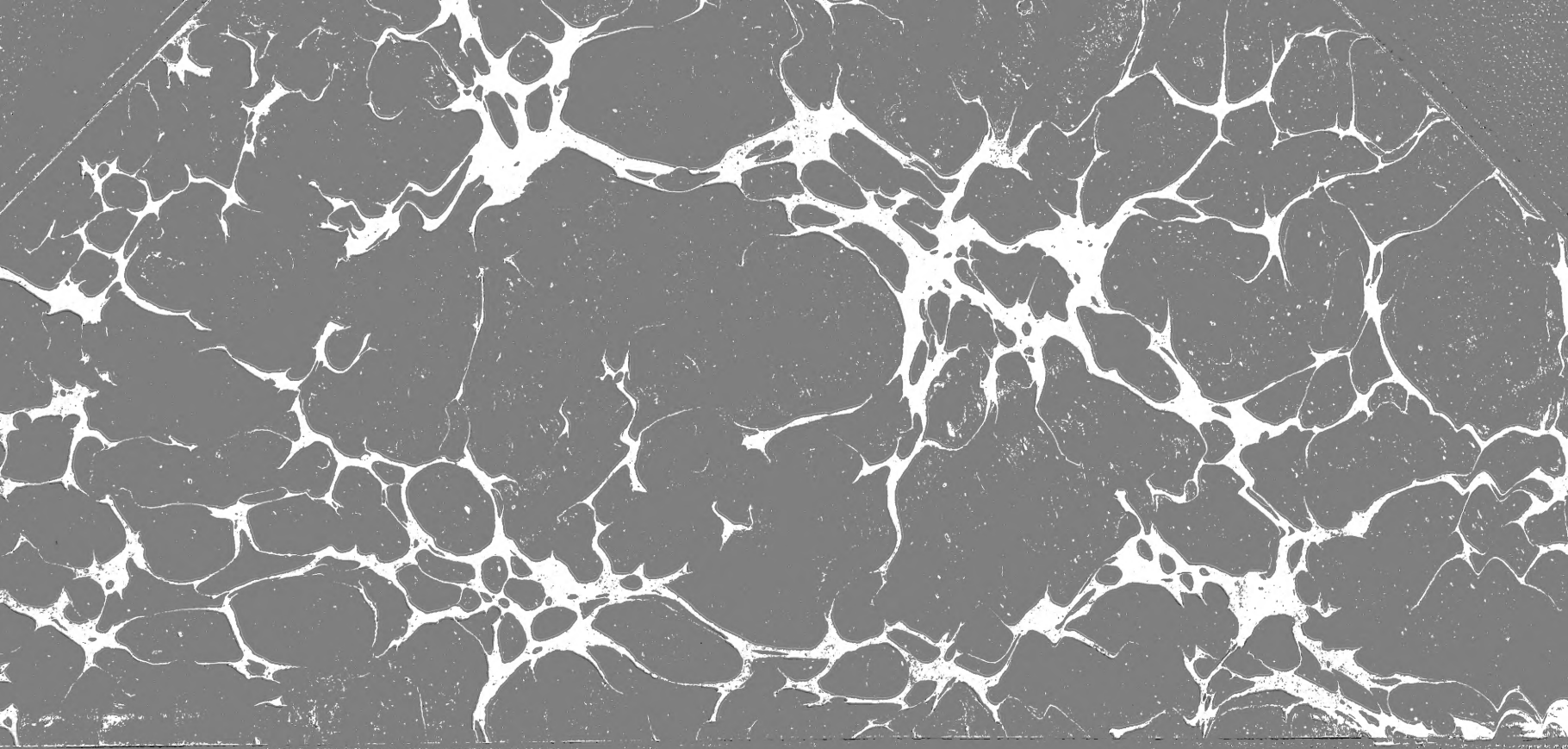

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OF

MICROSCOPICAL SCIENCE.

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SIR RAY LANKESTER, K.C.B., M.A., D.Sc., LL.D., F.R.S.,

HONORARY FELLOW OF EXETER COLLEGE, OXFORD; CORRESPONDENT OF THE INSTITUTE OF FRANCE, AND OF THE IMPERIAL ACADEMY OF SCIENCES OF ST. PETERSBURG, AND OF THE ACADEMY OF SCIENCES OF PHILADELPHIA, AND OF THE ROYAL ACADEMY OF SCIENCES OF TURIN; FOREIGN MEMBER OF THE ROYAL SOCIETY OF SCIENCES OF GÖTTINGEN, AND OF THE ROYAL BOHEMIAN SOCIETY OF SCIENCES, AND OF THE ACADEMY OF THE LINCEI OF ROME, AND OF THE AMERICAN ACADEMY OF ARTS AND SCIENCES OF BOSTON; ASSOCIATE OF THE ROYAL ACADEMY OF BELGIUM; HONORARY MEMBER OF THE NEW YORK ACADEMY OF SCIENCES, AND OF THE CAMBRIDGE PHILOSOPHICAL SOCIETY, AND OF THE ROYAL PHYSICAL SOCIETY OF EDINBURGH, AND OF THE BIOLOGICAL SOCIETY OF PARIS, AND OF THE CALIFORNIA ACADEMY OF SCIENCES OF SAN FRANCISCO, AND OF THE ROYAL ZOOLOGICAL AND MALACOLOGICAL SOCIETY OF BELGIUM; CORRESPONDING MEMBER OF THE SENKENBERG ACADEMY OF FRANKFURT-A-M; FOREIGN ASSOCIATE OF THE NATIONAL ACADEMY OF SCIENCES, U.S., AND MEMBER OF THE AMERICAN PHILOSOPHICAL SOCIETY; HONORARY FELLOW OF THE ROYAL SOCIETY OF EDINBURGH; LATE DIRECTOR OF THE NATURAL HISTORY DEPARTMENTS OF THE BRITISH MUSEUM; LATE PRESIDENT OF THE BRITISH ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE; LATE FULLERIAN PROFESSOR OF PHYSIOLOGY IN THE ROYAL INSTITUTION OF GREAT BRITAIN; LATE LINACRE PROFESSOR OF COMPARATIVE ANATOMY AND FELLOW OF MERTON COLLEGE, OXFORD.

WITH THE CO-OPERATION OF

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SYDNEY J. HICKSON, M.A., F.R.S.,

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AND

E. A. MINCHIN, M.A.,

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Introduction.

There are few more interesting unicellular organisms at present under investigation than those microscopic, yet most active, forms known as Spirochætes, which lie on the borderline between animals and plants. They are, indeed, veritable members of Haeckel's kingdom Protista, which—from their minute size and attenuate form—are most difficult objects for research. Little is known precisely of their structure, and their affinities are the subject of much discussion; hence the use of the term Protista in the preceding sentence. The wisdom of this classification for avoiding hard and fast lines where such do not naturally exist among the lowliest organisms, has now been recognised, and Schaudinn a few years ago founded a journal ('Archiv für Protistenkunde') exclusively devoted to the publication of memoirs on such organisms, including Bacteria. And the study of these forms is of the highest economic importance.

The famous French investigators, Laveran and Mesnil, regard these organisms as Bacteria, and in this view they are followed by Novy and his co-workers in America, who seem to regard the Spirochæte of human relapsing fever (S. obermeieri) as belonging to the genus Spirillum.

The late Dr. Schaudinn and the members of his school (for example, Prowazek, Hartmann, Keysselitz), on the other

hand, regard the Spirochætes as Protozoa, more especially on account of Schaudinn's remarkable memoir on the Trypanosomes and Hæmosporidia of the little owl (Athene noctua) and the occurrence of so-called Spirochæte stages. Schaudinn himself, however, modified his own views later, and acknowledged that Spirochæta ziemanni was really a thin elongate Trypanosome.

The type species of the genus Spirochæta (Ehrenberg) is S. plicatilis, founded by Ehrenberg in 1833, and obtained from muddy pond-water. According to Schaudinn (1905) this type-species possesses an undulating membrane. Strict members of the genus Spirochæta should, then, possess an undulating membrane, while the members of the genus Spirillum are characterised by the presence of flagella ("cilia" of French authors), especially terminal ones.

A closely related organism to these is Treponema (Spirochæta) pallidum (Schaudinn) from syphilitic lesions.

The generic and specific characters of these and related forms are in a most confused state. Even in memoirs by well-known medical authors we read of the "Spirochætes of Spirillosis," a phrase which must grate on the ear of both zoologist and bacteriologist. Before, then, any very definite advance can be made, it is necessary to obtain precise accounts of the movements and structure of some Spirochætes, irrespective of the inoculation experiments of the pathologist or the fanciful phylogenies of the compiler. Protistology, at this stage of its development, has—it seems to me—little use for the mere compiler, whose chief delight is in balancing the conflicting statements of others and submitting a new view of his own, sometimes even without seeing the specimen, which procedure only adds to the confusion. In other words, it is necessary to study living material with patience and determination, leaving, to some extent, the question of hypothesis or affinity as a sequel to the study.

Whilst working in France during July and August, 1906, I

was fortunately able to obtain a few oysters infected with Spirochæta (Trypanosoma) balbianii, Certes. The parasite occurred in the crystalline style, and those parts of the gut more especially around it, namely, the stomach, intestinal cæcum, and anterior part of the intestine proper. These researches were begun in the famous marine laboratory of Roscoff, Brittany, which I visited at the kind suggestion of Professor Minchin and through the courtesy of Professor Delage, of the Sorbonne. At this laboratory I was enabled to study this most interesting Protist at first hand. I would take this opportunity of returning my best thanks to Professor Delage and the staff of the laboratory at Roscoff for their kindness and attention, also for their hospitality in the form of free rooms while working there. To M. Fred Vlès, Préparateur du Laboratoire, I am especially indebted, as it is owing to his researches on Lamellibranchs that oysters, some of which proved to be infected with Spirochætes, were introduced into the laboratory at Roscoff, having been obtained from Abervrach.

The research on this and allied organisms has been continued since in London on infected material procured after much difficulty.

In an appendix to a paper by Prowazek, published in 1906, Keysselitz (**4**) notified the occurrence of a new Spirochæte (S. anodontæ) from Anodonta mutabilis. His description is somewhat meagre, and the dimensions of the parasite are not given. Anodonta mutabilis is not a British species. After dissecting specimens of Anodonta cygnea, the well-known British fresh-water mussel, from different localities, I was able to announce[1] the occurrence of a Spirochæte therein, probably the S. anodontæ of Keysselitz, for it possesses pointed ends, as stated by him; and I am able to supply details of its movements and structure. I have much pleasure in thanking Mr. O. H. Latter, of Charterhouse, Godalming, for specimens of A. cygnea containing these Spirochætes, and for his excellent account of

[1] 'Proc. Zool. Soc.,' May 7th, 1907.

the morphology of the mussel (**27**), wherein he mentions the occurrence of "spirilliform bacteria" in the gut of Anodon.

Historical.

Spirochæta (Trypanosoma) balbianii was first recorded by Certes (**1**) from French oysters in 1882, though Möbius (**7**), writing in 1883, states that he observed the parasites in 1869 in the oysters of Schleswig-Holstein. Certes gave very good figures of the organism, considering the date and instruments of research then available for the study of such minute forms. He placed it, more especially on account of its possession of an undulating membrane, in the genus Trypanosoma, at that time ill-defined and little understood. Lustrac (**6**) in 1896 described longitudinal division and the rôle of the membrane therein. In 1901 the famous French protistologists, Laveran and Mesnil (**5**), briefly described the main features of the organism, and stated that it was really a Bacterium allied to the Spirochætes and Spirilla. This paper was, and, in my opinion, still is, the most important contribution to the structure and affinities of the organism. Spirochæta balbianii has no flagellum; yet, in spite of this, Perrin, in 1905–6 (**8**, **9**), still retained the parasite in the Trypanosomidæ, which seems to me most distinctly to be a retrograde proceeding. Perrin, however, made many excellent observations on the Spirochætes obtained from Adriatic oysters, but seems to have allowed his judgment to have been influenced, at any rate to some extent, by a preconceived idea of the "Urhaemoflagellat." A few other short notes by Vlès (**11**), Swellengrebel (**10**), and Fantham (**3**) have appeared recently, but no detailed accounts of the movements of the animal, the structure of the membrane, or a broad discussion of the systematic position of the organism have yet been published. Disputed points are whether the membrane is really such or only a sheath ("gaine" Laveran et Mesnil), whether encystment takes place or not (Perrin), and whether so-called

polymorphism occurs; while the precise method or methods of division also require re-investigation. I hope to deal with these questions in the sequel, but the Spirochætes—on account of their minute size and active movements—offer the very greatest difficulties as objects of research, and, in endeavouring to elucidate some of these difficulties, one must try to preserve an open mind, and not be dogmatic.

Regarding Spirochæta anodontæ, as stated already, this form was discovered and briefly described by Keysselitz (4) in 1906 in the crystalline style of Anodonta mutabilis. Its occurrence in the British mussel, A. cygnea, was not recorded previously to my note before the Zoological Society. I hope in the following to set forth some details regarding its movements and morphology.

Material and the Occurrence of Parasites therein.

I think it would be well to treat together of the material used and the occurrence of the parasites therein, and not to take these sections separately as was at first intended. Oysters and fresh-water mussels are common enough, but infected specimens are not by any means plentiful in either case. The material for the study of Spirochæta balbianii was obtained alive in Roscoff from oysters imported from Abervrach, and later in London from English native oysters.

Many samples of English oysters were tried, for example, from Whitstable direct, Brightlingsea, Shoreham, and other places, sometimes unknown. The infected specimens were bought at Billingsgate, and were stated to be English natives, but that was all the information vouchsafed, though others can be got from the same bed. There have been several "oyster scares" lately, and dealers are somewhat reticent.

The infected oysters were rather small and came from very muddy beds, and all who saw them remarked that they did not look very palatable. The oysters were kept alive in sea water for the space of a fortnight. This water was constantly changed, and was sometimes previously filtered.

With regard to Spirochætes in oysters there is a great

difficulty at the outset. The habitat of these parasites is in the anterior part of the alimentary canal, especially in the crystalline style, and occasionally in that part of the liver bordering on the stomach. Now the crystalline style very soon dissolves and disappears when the oyster is taken from the sea. Even when kept in an aquarium connected with the sea, as at Roscoff, the number of oysters possessing crystalline styles, when dissected, is low. A French Professor of Zoology informed me that he once left Roscoff with specimens of a strain of oysters known to be infected with Spirochætes, and, after travelling in the train to the south of France, found, on arrival at his destination, that the style had dissolved, and that the Spirochætes were in most cases dead. There seems no doubt whatever that to find the Spirochætes plentiful and active in the crystalline style—which is probably their principal natural habitat—one must take the oysters from the sea and examine them at once. Perrin also comments on this fact.

Yet, on the other hand, Laveran and Mesnil were able to find Spirochætes in oysters freshly procured in the market of Paris. But Paris is favourably situated with regard to the supply of oysters.

In my own case, of the specimens bought in the London market, about 50 to 60 per cent. of the infected strain actually contained parasites. Of these oysters, comparatively few, less than 5 per cent., contained crystalline styles. The parasites, however, were chiefly confined to the regions occupied by the style and surrounding structures, namely, the stomach, anterior part of the intestine, and the so-called cæcum. The latter in the oyster openly communicates above with the intestine, but is partially separated off by a ventral upgrowth or fold of the wall of the gut, a sort of "typhlosole." No parasites were found actually in the substance of the "flêche tricuspide" of Poli, which is a cuticular lining of the stomach. Few parasites were found in the posterior part of the intestine or in the rectum.

I am inclined to think that parasites swimming about freely

in the gut are somewhat thinner than those normally occurring in the crystalline style.

In the case of Spirochæta anodontæ the infected mussels nearly always contained crystalline styles, in which the parasites could be descried actively swimming. About 80 per cent. of the mussels were infected.

The position and connection of the crystalline style of Anodon have been well described by Mitra (**28**), except that his figure of the natural size of the style is quite wrong. This structure is usually 5 cm. to 7 cm. in length, and 0·5 cm. to 0·7 cm. broad; at any rate those are the dimensions I found after careful measurement of numerous specimens. It is irregular at the anterior end, but tapering at the posterior one. It is not definitely joined up to the cuticular lining ("flêche tricuspide") of the stomach, but a granular loose coagulum often connects them. This structure is discussed more fully in Appendix I, and is only mentioned here in connection with the occurrence of the parasites therein. I have sometimes found S. anodontæ in the "flêche tricuspide" of Anodons from Godalming, but the Spirochæte always occurred in the crystalline style. I never found S. balbianii in the "flêche tricuspide" of Ostrea, whether the oyster was infected or not, and Perrin mentions that he never found it there; at least, I think that it is the "flêche tricuspide" that he really means when he remarks: "In the anterior part of the stomach is another structure, the meaning of which I am unable to explain. This structure is connected with the anterior end of the style by a dark-coloured mass of diatoms. It is an irregular mass conforming to the shape of the anterior region of the stomach, is transparent and crystalline, and of a firmer consistency than the style itself. It is very regular in its occurrence, and after prolonged periods of starvation still appears to retain its place. It contains no Trypanosomes" (**9**, p. 134).

The Spirochætes swim about most actively in the colloidal substance of the style in all directions and planes, often suddenly reversing their path of motion.

It may be mentioned that the reproductive organs of the oyster (**25**), and of infected Anodons were examined by me, but no Spirochætes were found therein, although Koch (**16**) found S. obermeieri in the ovary and ova of the tick. It is quite possible that the oysters examined by me were not mature.

Specimens of Atax bonzi, Clap. (**26**), occurring in the mantle-cavity of Anodonta cygnea, probably as commensals (**27**), were found to contain no Spirochætes, though S. duttoni occurs in ticks (**14a**). But S. anodontæ is a parasite of the gut, and probably primitive in its habitat.

Methods.

(*a*) For Fresh Material.

I have made a special point of examining these two Spirochætes in their natural environment as far as possible. This method does not appear to have been followed in the case of S. balbianii to any great extent by previous observers, Perrin excepted, judging by their published statements, while there is no account of an examination of S. anodontæ in the living state.

When a style was present the freshly extracted structure was mounted in a drop of sea water or fresh water in the case of Ostrea and Anodonta respectively, and placed in a moist chamber. The organisms were often thus kept alive from three to six hours, while the style was examined in sections in the laboratory at a temperature above that of the sea or the fresh water in which the hosts lived.

In the case of portions of the style the inner fluid contents were pressed out as far as possible with a fine scalpel held flat, or with a triangular-headed lancet as recommended by Perrin. The outer and firmer part of the style, to which the parasites often cling for a time, may, after a while, be separated out of the preparation if possible. But on the whole it is best to fix the smear wet with osmic vapour as quickly as possible.

At other times hanging drops of the parasites in their natural medium were made and examined. Such preparations, securely vaselined round the edges, were carefully observed under the microscope. Similar preparations of the gut contents were placed under a cover-glass supported on the slide by wax feet at the corners, and then vaselined round the edges.

Methylene blue was tried as an intra vitam stain and was found successful. The Spirochætes, while still alive, stained deeply with a half-per-cent. solution of this dye in sea water or fresh water as the case might be, but usually died in about half-an-hour. Near the edges of the preparation, or in a part where the methylene blue solution (after being run under the cover-slip or added to water containing the parasites) seemed less concentrated, there the Spirochætes remained alive some time longer; but they were not very active under these conditions.

Examinations were made, from time to time, of the media in the aquaria or basins in which the Lamellibranch hosts were kept during these investigations. In this connection it is interesting to be able to record that Spirochæta balbianii was found moving about freely in two different basins of sea water in which specimens of infected English oysters were kept, the shells of these being still partly covered by mud from their native beds. I was able to demonstrate this, with living specimens, to some of my friends.

These various preparations were observed under Zeiss AA, Zeiss DD and one-twelfth inch oil-immersion objectives, with Huygenian eye-piece 4. In the case of S. balbianii the one-sixth inch (Zeiss DD) was found most useful, for the parasites move very rapidly and over a large field. It is rather difficult to keep the organism under the comparatively limited field of an oil-immersion lens and, of course, the working distance is small.

However, such an examination was conducted, but it added little to the observations made with a Zeiss DD lens. A similar procedure was followed in the case of S. anodontæ,

which moves more rapidly, and so is difficult to keep in the field.

In the case of each of the Spirochætes mentioned, the thickened border of the membrane could be observed in life, with comparative ease in S. balbianii, with greater difficulty in S. anodontæ. The spiral movements, and the undulations travelling down the body of the parasites while in active motion under the practically normal conditions, were thus observed. But these movements will be described in detail in the next section.

I have also postponed all consideration and discussion of environmental effects and attempts at cultures of these parasites until after my description of the normal forms, as seen in both living and fixed preparations.

(*b*) For Fixed and Stained Material.

Many methods of fixation and subsequent staining were tried, and the resulting appearances of the parasite were always carefully compared with those seen in life under normal conditions as far as possible. This procedure was adopted in order to eliminate, if possible, deceptive appearances or artifacts due to pathological changes or post-mortem effects. Where such abnormalities occurred allowances were made for them, as judged by observations on the actively living parasitic organisms. In this way an interpretation can be given of so-called flagellate or ciliate stages in Spirochætes (see p. 32).

Fixation.—Thin smears or films were made from the gut contents of the Lamellibranch hosts. The contents were taken up with a pipette and placed quickly on a slide, or a part of the infected crystalline style was quickly teased with needles while kept moist in a little of its natural surrounding medium. Such preparations of gut contents, usually on slides or at other times on cover slips, were fixed, as quickly as possible, and while still wet, with osmic vapour. The spreading or thinning out of the film was

sometimes performed before fixation with osmic vapour, while on other occasions the spreading was deferred till after fixation. The latter plan was probably the better. The drop of liquid containing the parasites was held, attached to the under surface of a carefully cleaned slide, over the mouth of a bottle containing osmic acid in aqueous solution of 2 to 4 per cent. strength, and fixed for one to four minutes. The adherence of the film to the slide, in these cases, was secured by the albuminous material naturally occurring in the contents of the alimentary tract of the Lamellibranchs. At other times albuminised slides were used, and the drop of liquid containing the parasites was put thereon before fixation was attempted. In this way smears containing the parasites were placed, with the smear side downwards, in Flemming's solution, in Hermann's solution, or in a mixture consisting of two parts of saturated aqueous corrosive sublimate to one part of 90 per cent. alcohol.

Of the various fixatives used I preferred osmic vapour, next mixtures containing osmic acid, and lastly the sublimate-alcohol.

Dried smears, the fixation of which was afterwards completed just before staining with pure methyl or pure ethyl (absolute) alcohol, also gave, on many occasions, very good results. For the structure of the delicate membrane of the parasites, however, osmic acid was undoubtedly the best fixative, but absolute alcohol also revealed the same.

Another method of smear preparation was also tried, which is really a variation in the manner of securing adherence to the slide. A drop of the gut contents or a portion of the dissolved style was first fixed with osmic vapour before being spread, and then mixed with a drop of glycerine and albumen. This glycerine and albumen mixture is that usually employed for securing the adherence of sections to slides. After this the drop was immediately spread, and so a thin and even film was obtained.

To sum up regarding fixation, I obtained excellent results from the action of osmic vapour on rapidly prepared fresh

films. I found it often unnecessary to add artificial media, like glycerine and albumen, even after fixation, and thus avoided the risk, however slight, of artificial effects due to the added media, for these parasitic organisms are very small and delicate.

Stains.—Of the various stains tried on fixed material, prepared as above described, the most successful results were obtained with alcoholic gentian-violet (Ohlmacher's formula, containing a little formalin), iron-alum hæmatoxylin, Delafield's and Ehrlich's hæmatoxylins, Giemsa's stain, and Billet's modification of the latter (that is, carbonated blue added to azur II and eosin). Leishman's stain was used a little while in France. Alcoholic safranin and Löffler's methylene blue were also good, while thionin and basic fuchsin were fairly useful. I was not very successful in my attempts with a dilute aqueous solution of silver nitrate.

Tannin orange was often found most useful after Giemsa's or Billet's stains, and also on those occasions on which Laveran's mixture of Borrel blue and eosin was used. Tannic acid itself was tried to show the possible presence of flagella, but without result. "Acid plasma stains," like Orange G or Säurefuchsin, were found to be of little use, if not positively harmful, after hæmatoxylin.

The most difficult structure to stain was the membrane. For revealing new details in this "organella" gentian-violet and iron-hæmatoxylin were most useful, especially the former in the case of S. balbianii. Hæmatoxylin has not, I believe, been used in investigating these two organisms before, though it gives very good results. Staining for a long period is necessary to show clearly the presence of the membrane, and, in the case of Delafield's or Ehrlich's hæmatoxylin, this is fortunately not accompanied by over-staining of the nucleus.

The various modifications of the Romanowsky coloration usually over-stain the internal structure of the parasite before staining the membrane, and the striated character of the membrane is seldom, if at all, clearly brought out by these Romanowsky stains. It is probably on

this account that previous observers have failed to notice this feature in S. balbianii. I am convinced that there is a great tendency to attach too much importance and reliance to the Romanowsky coloration in protistological work, to the exclusion of the older and well-tried stains like hæmatoxylin. But one needs to consider and correlate the results obtained by all of these stains after various fixatives by careful comparison and testing with observations on living material.

With such small organisms it is difficult to differentiate when using iron-hæmatoxylin. Fixed and stained preparations were mounted in cedar-wood oil or in Canada balsam. Unmounted preparations were also examined.

Sections.—A piece of crystalline style of Anodon, known to be infected, was fixed in Flemming's solution, embedded in paraffin, and sectionised. The sections were stained with hæmatoxylin, Giemsa's solution, iron-hæmatoxylin, and methylene blue. In this manner various sections of Spirochætes were obtained and examined under the microscope, and a knowledge of their internal organisation was thus gathered with some difficulty (see text-fig. 6).

General Structure.

It would be well, I think, to give at this point a brief outline of the chief morphological features of these two Spirochætes before discussing their movements, though the movements of the living organisms should, it seems to me, be considered before a detailed examination of structures observed in fixed and stained preparations is undertaken.

Spirochæta balbianii is a long, sinuous, thread-like organism about 50 μ to 150 μ in length, and 2 μ to 3 μ in breadth. It consists internally of homogeneous protoplasm and a diffuse nucleus in the form of small, transversely-arranged rods of chromatin, about sixty in number, disposed at nearly equal distances along the body. Perrin has given to these rodlets, which are sometimes dumb-bell shaped, and exhibit other slight variations in size and form, the name of

"chromosomes." However, as was kindly pointed out to me by Professor Ray Lankester, the use of the term involves an assumption, for the nucleus in these Spirochætes is diffuse, rather like the conditions occurring in the Bacteria. It does not, then, necessarily follow that these rodlets of chromatin, which are numerous and not easily seen, are strictly homologous with the chromosomes of the concentrated nuclei of the Metazoa.[1] Further, in carefully and successfully stained preparations, it is seen that these chromatin rodlets are disposed on a more faintly staining spiral or zig-zag structure, the so-called "karyosome" of Perrin, so that they may be only large chromidial granules.

The cytoplasm of these Spirochætes is, as previously stated, apparently homogeneous and hyaline. It is not easily differentiated into ectoplasm and endoplasm, though in a few cases a slightly more granular endoplasm could, perhaps, be detected round the nucleus. The outer or ectoplasmic layer of the cytoplasm forms a definite cuticle or periplast, giving a distinct contour to the organism. The ends of the body in S. balbianii are rounded, as in the case of the type-species S. plicatilis. On the other hand, in S. anodontæ they are pointed, a feature of the greatest interest, as I hope to show in the sequel.

Perhaps the most interesting structural feature, or "organella," possessed by the Spirochætes is a spirally wound membrane arising as a lateral outgrowth of the periplast, and extending nearly from end to end of the body. It has been called an "undulating" membrane. By means of special stains, such as gentian-violet and iron-hæmatoxylin, this membrane is seen to be approximately longitudinally striated, a feature not before recorded in these parasites. The question of the structure and significance of this "organella" is of the greatest importance, and will be considered in detail in a later section.

[1] Sir Ray Lankester has since suggested to me that the term "nucleoid" would more correctly describe the condition of the chromatin in forms like Spirochætes than the term nucleus does. Owing to the numerous alterations

S. anodontæ is usually about 40 μ long and 0·7 μ broad.[1] Its ends are pointed, and it possesses a spirally wound undulating membrane, and a diffuse nucleus of chromatin rodlets arranged on a more faintly staining spiral.

Movements of these Spirochætes.

Previous accounts of these phenomena are most meagre; yet descriptions of such movements are much to be desired, and would be of the utmost importance in helping to decide, for example, whether the pathogenic organism, S. obermeieri, of human relapsing fever is really a Spirillum or a Spirochæte. I will now attempt to supply this omission, to some extent, by giving as full account as I can of the movements of S. balbianii and S. anodontæ.

This is, however, a question of great complexity, and the movements are most difficult to analyse and interpret, both on account of the rapidity of the motion and of the smallness and extreme tenuity of the organism. One naturally turns for aid in solving the problem to slowly moving and comparatively quiet forms, or to forms partially entangled among débris for a short time, but it must be constantly borne in mind that the movements of such organisms may not be quite normal.

S. anodontæ moves rather more rapidly than S. balbianii, indeed, so quickly that it is almost impossible to analyse its motion when travelling at full speed, and each of them moves faster than most Trypanosomes. The movements are much the same whether the organism be examined inside the crystalline style or moving freely in the lumen of the gut.

The path may be either in a straight line or more or less in

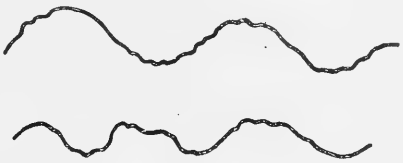
involved, the term was not substituted in the body of the text, but it would have been preferable to have used it throughout the memoir.

[1] Specimens vary from 30 μ to 60 μ in length, and from 0·7 μ to 1 μ in breadth.

a circle, while motion along paths forming complex geometric figures, as helices, figures of 8, and Catherine wheels also occurs (text-fig. 4, and Pl. 1, fig. 6). These figures are often of great beauty in spite of their complexity, and an organism executing them is most interesting, yet bewildering, to watch. Lashing movements are also seen sometimes.

In the case of slowly moving specimens it is seen that the organism moves forward while turning on its long axis.

The motion appears to be resolvable into at least two components: (i) An undulatory flexion of the body, mainly for progression, and (ii) a spiral or corkscrew movement of the body as a whole, due to the winding of the membrane. The corkscrew motion is especially well seen in the case of



Text-fig. 1.—The outline of the sinuosities of the moving organism is a little irregular. The contour of the waves is broken by smaller waves, though in the lower figure this is somewhat exaggerated.

S. anodontæ, and is probably to be correlated with its possession of pointed ends. The spiral motion, in the case of S. balbianii, is not at first sight very apparent in all specimens, and its ends are blunt.

Waves can be seen travelling down the thread-like body in a direction opposite to that in which the organism is progressing. Many waves or sinuosities, some eight or ten, or even fourteen, can be seen along the body of rapidly moving forms, while only some two to four may occur along the body in more slowly moving organisms. The outline of the sinuosities is sometimes a little irregular—that is, the contour of these waves is somewhat broken by much smaller waves (text-fig. 1).

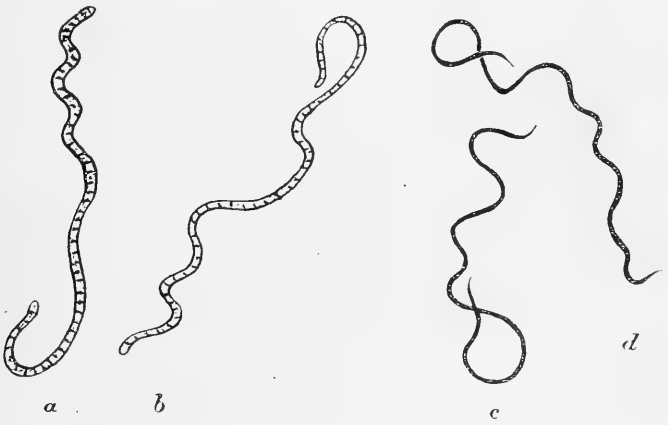
The movements occur in jerks. The organism may suddenly

come to a dead stop or just as suddenly proceed more slowly. It is a matter of indifference which end of the body is directed forwards, for the parasite is capable of suddenly reversing its direction of movement and returning on its own path, apparently in an almost exact straight line or circle. I do not consider this retracing of its path to be due to unfavourable environment, as suggested by Novy in the case of S. obermeieri, for I have observed it constantly taking place inside the crystalline style. The organism can then travel with or against the current indifferently. A very great deal of energy seems to be used in the motion of the organism. The body of the parasite can be distinguished during motion in the case of S. balbianii with some difficulty, but sometimes S. anodontæ moves too rapidly for its outline to be discernible. However, in the case of S. balbianii, I do not agree with Perrin when he remarks: "When liberated from the crystalline style into sea water, motion is very fast, . . . The body itself cannot be seen. Two black points, the anterior and posterior ends of the animal are alone visible." When moving in this manner, which, I take it, is in a plane approximately that of the preparation and along the stage of the microscope, I was always able to see the outline of the body, however fast the organism moved. A somewhat blurred appearance might occasionally arise, even in the "side-to-side" movement of the whole body, quoted by Perrin, but even then the organism, as a whole, was visible. The ends in ordinary progression do not lie in a different plane from that of the intermediate parts of the body, nor do they appear to be especially fixed, for if one end seems somewhat fixed the other one often moves from side to side. In rapid forward motion, then, the body seems to be thrown into undulations travelling down the body from the anteriorly directed end to the posterior one, while the body rotates on itself in a spiral manner, and the side-to-side movements or lashing of one end are reduced to a minimum at this time.

Sometimes the organism appears suddenly from a deeper level of the liquid under examination and swims, or rather,

spirally bores its way more or less vertically upwards. In this condition it twists itself into various peculiar shapes, and so resembles a Catherine wheel as described by Perrin (text-fig. 4).

Parasites are sometimes noticed anchored by one end to débris, such as a detached epithelial cell of the gut of the host, now lying free in the gut contents (Pl. 2, fig. 28). The free end of the parasite then executes violent lashing movements or intermittent flickers, not unlike those of a



TEXT-FIG. 2.—The free end of an anchored parasite or of a very slowly moving form may curl over. *a*, *b*. S. balbianii. *c*, *d*. S. anodontæ.

"flame cell." The free end also in such specimens may coil itself over and over (text-fig. 2; also Pl. 1, fig. 1; Pl. 2, fig. 12; Pl. 3, fig. 21). S. balbianii, with its rounded ends, often has some difficulty, apparently, in boring its way through débris or obstacles in its path. It often tries to get through these instead of changing its direction of motion. However, I have seen it penetrate free epithelial cells and occasionally appear to come out of such cells. S. anodontæ, with its pointed ends, succeeds rather better on such occasions, and more easily bores its way through or among the débris of cells and diatoms floating freely in the gut-contents of the host.

Spirochætes may at times be seen vibrating in two halves about their central points as nodes; they then bear a general resemblance to two tuning-forks joined by their single ends and in vibration.

Slowly moving specimens of S. anodontæ may curl up one end (Pl. 3, fig. 35; also Pl. 2, fig. 33, and text-fig. 2), usually the hinder one, judging by the direction of motion. This may be carried still further in the case of very slowly moving specimens where each end of the organism coils up, and one gets figures like that shown in text-fig. 3, where two loosely coiled watchspring-like curves face each other, and are conjoined in the middle (cf. also Pl. 3, fig. 36).

These Spirochætes seem to move more quickly than Try-

Text-fig. 3.—Outline of very slowly moving specimens of S. anodontæ, with each end coiled.

panosomes, and with an added corkscrew motion. I am aware that Trypanosomes sometimes turn over during movement, but the membrane in the case of these undoubted Flagellates is a strict lateral outgrowth of the body, and not spirally wound, while there is a long, free flagellum. The membrane also vibrates in the case of Trypanosomes, while there is little of this vibratory movement connected with the membrane of Spirochætes. I consider that the membrane of Spirochætes is attached to the body in the form of a spiral, or, more precisely, that it is a spirally arranged lateral extension of the periplast, and that its spiral winding is not merely due to a torsion of the free part of a lateral membrane, which may untwist (as Woodcock seems to suggest [**24**, p. 221]). The constant spiral movement of Spirochætes, I think, bears out the correctness of the view herein set forth.

Comparing the movements of Spirilla with those of Spirochætes, it may be remarked that the body of a Spirillum while in motion seems more rigid than that of a Spirochæta, and, of course, flagella are present in the case of true Spirilla. I have carefully noted the movements of Spirilla occurring in the hind-gut of the Cockroach for comparison with those of Spirochætes.

Various scientific workers—both zoologists and bacteriologists—to whom I have shown these Spirochætes alive, have compared their motion to that of an eel, the embryo of Filaria, or Nereis, but with the spiral movement in addition.

The Spirochætes live by endosmosis in the fluids in which they swim, and the membrane—although principally an agent of movement—may serve to increase the general absorptive area of the body.

The Mechanism of Movement in the Spirochætes.

The subject of the agency and mechanism of movement in the parasitic Protozoa is most difficult, and seems to have been generally avoided by most authors—indeed, the whole question of the movements of Trypanosomes, Spirochætes, sporozoites and the like, appears to have been little studied or described. Regarding the movements of Gregarines and their causation we have the accounts of Schewiakoff and Crawley, of those of Trypanosomes by Laveran and Mesnil, of Coccidian sporozoites by Schaudinn, and the meagre account of the movements of "Trypanosoma" balbianii by Perrin. These are amongst the principal accounts on this subject, and some of them are not very detailed, nor do they go to the root of the question.

This is a problem of the utmost difficulty, and the examination of stained preparations, to which so much present-day research is often restricted, sheds little light on the matter.

As a result of much patient observation on living material

and much cogitation on the question, I will now endeavour to set forth, not without some diffidence, an interpretation of the mechanism of the movements of Spirochæta balbianii and S. anodontæ, together with, and supported by, certain (unpublished) observations, also made at Roscoff, on the movements of living Gregarines (Selenidiidæ) of the gut of Phascolosomes (P. vulgare and P. elongatum).

My observations on the movements of the trophozoites of the Selenidiidæ of Phascolosoma, in brief, entirely support the views of Crawley as to the movements of Gregarines. I saw a slight quantity of gelatinous material extruded from the Gregarines as described by Schewiakoff, but I cannot believe that such is sufficient to account for the gliding movement of the vermiform trophozoites. In the Selenidiidæ the longitudinally arranged myocyte fibrillæ or myonemes are well marked, and, by their contractions, set up pressures in a plane at right angles to the long axis of the body. One had ocular demonstration of the result of these pressures, for, under favourable conditions, I have seen even the somewhat spherical nucleus of the Gregarines altered in shape and rendered more transversely ovoid, and vice versâ.

Now, it seems to me that these observations have a direct bearing on the movements of Spirochætes, though the velocities of forward movement of Spirochætes and Gregarines are very different, the latter being much slower than the former. I have already mentioned the occurrence of myonemes in the spirally wound membrane of Spirochætes. These myoneme fibrils, by their shifting or movement of pressing inwards or outwards towards the attenuated cylindrical body, set up transverse movements in the periplast surface—probably as alterations in the position of the striations of the periplast membrane and body generally—in a plane at right angles to that of the long axis of the body, which axis is also that of the forward direction of movement. An impulse of this kind, starting at the anteriorly directed end, sets up a wave passing backwards towards the hinder

end. This wave can be seen passing down the flexible body in a direction opposite to that of the forward motion of the organism, and a return wave is sometimes visible in favourably placed specimens.

The occurrence of specimens with membranes loosely attached to the body, while others have membranes closely attached and contracted against the body (see Plate 1, figs. 5 and 8), supports the view of transverse movements of contractile myonemes and the waves along the body set up thereby. The onward gliding movement of the Spirochætes is thus accounted for. Its concomitant spiral rotation is, to my mind, obviously due to the spiral winding of the membrane which directs the long, thread-like body torsionally, thus guiding its movements in a spiral or corkscrew manner.

The membrane itself does not undulate to any great extent. This I very soon noticed in my examination of living S. balbianii, and some of my friends who have seen the organisms alive have also remarked on this fact.

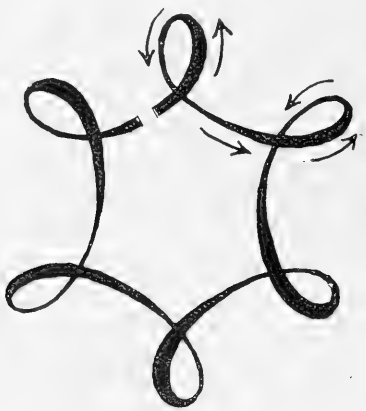
I prefer, then, the term "membrane" to that of "undulating" membrane in connection with Spirochætes, thereby emphasising a contrast with the "organella" of Trypanosomes, which truly vibrates, and may be styled correctly an "undulating" membrane.

The lashing movements of these Spirochætes, together with the curling up of the posterior or free end—especially marked in S. anodontæ—I ascribe to alternate contractions and relaxations of the myoneme fibrils of the periplast of the thread-like body.

In connection with the undulations passing down the body it has been mentioned already that there is a feeble return wave ("reaction-wave"), sometimes quite easily discernible, which starts from the posteriorally directed end, and passes forward. At the moment of reversal the transversely directed inward impulse suddenly begins from the opposite end, which is the former posteriorally directed one, and this change in the point of application of the greatest transverse pressure brings about the reversal of motion. It is, as it were, that

the return wave (previously mentioned) suddenly becomes the new principal wave. In other words, the whole process is merely a question of transverse pressure, which is first applied at one or other of the ends of the organism, and sets up waves travelling from end to end. Also the point of application of the principal transversely applied inward pressure may be suddenly altered, for the intensity of the force and its point of application may alternate from end to end.

In the case of lashing movements probably the myonemes



Text-fig. 4.—"Catherine wheel" figure, executed by Spirochætes on occasions of exaggerated spiral motion—"end-on" view.

of the body surface are the principal agents, while those of the membrane probably dominate in the ordinary progressive movements of the organism.

The exaggerated spiral movements of S. balbianii (and the same applies to S. anodontæ) were first mentioned by Perrin, and compared with the rotation of a Catherine wheel (text-fig. 4). These must result from an "end-on" view of the organism progressing upwards or downwards in a plane more or less at right angles to that of the stage of the microscope. I rather think that the organism is "not very happy" under such circumstances, and that the spiral move-

ment in these cases is amplified into the abnormal or nearly so.

Perrin considers that the membrane is only of secondary importance in the mechanism of movement, for he states that "there is but little noticeable difference in the motion of forms with or without it." Now there is probably no form of S. balbianii (or S. anodontæ) devoid of a membrane, and in those organisms apparently without it the structure is really present and closely contracted round the body, as shown by its deeply staining border being seen as a sinuous line along the outside of the periplast in all such when successfully stained (vide Pl. 1, figs. 4, 7; Pl. 2, fig. 34; also Pl. 3, fig. 23).

It might, perhaps, be urged by the hypercritical that the undulations seen passing along the body in motion are only "optical illusions" due to the organism turning round on itself, and so bringing different points of the body into focus under the microscope. I do not think so myself. I hope I have shown, in this section of the memoir, the necessity for the presence of these waves in aiding in the motion of the organism. Also, in carefully fixed and stained preparations, the body is actually seen fixed in the form of waves, as a sinuous thread; in fact, rigid and straight forms of Spirochætes are hardly ever encountered, for such, if persistent, would belong more properly to the genus Spirillum. Optical illusions cannot be stained so as to show when illuminated by a wide-angled central pencil of light.

The central nuclear thread is, I think, merely flexible, that is, able to be bent into the form of the waves set up by the myonemes. I do not consider the nuclear thread to be contractile (other than possessing the general "tonic contractility" in common with all forms of protoplasm), as is, perhaps, suggested by Swellengrebel (**10**). But really he may mean the chromatin border of the membrane, which certainly is contractile—his context is a little vague on this matter.

Detailed Morphology.

As stated, the attenuated body of these organisms consists of a clear cytoplasm surrounding a spiral granular band of chromatin. The cytoplasm usually appears homogeneous, but in favourable specimens an inner, slightly more granular layer can be seen, which is the endoplasm and surrounds the nucleus.

Cytoplasm.

The outer layer or **ectoplasm** is clear, and generally entirely converted into a periplast. In some specimes of Spirochæta balbianii kindly stained for me by Dr. A. C. Stevenson with Billet's modification of the Giemsa stain (namely, carbonated blue added to methylene-azur and eosin), the outermost layer of the body, that is, the external portion of the periplast, is coloured red, and slight dots appear along it (Pl. 3, fig. 19, *my.*), giving it a beaded appearance. These dots may be merely a staining artifact, but personally I think they are not artifacts, but represent a normal structural feature of the organism, namely, the presence in the outer portion of the periplast of contractile fibres (myocyte fibrillæ or myonemes). Dutton and Todd (quoted in [**24**, p. 210]) mention an apparent ectoplasmic structure in Trypanosoma mega staining pinkish with the Romanowsky stain, and showing myoneme structure. I think there is a myoneme-containing layer in the periplast of Spirochætes (vide Pl. 1, figs. 2, 5*a*, 11, *my.*), and the dots (Pl. 3, fig. 19) found therein—giving it a beaded appearance—may be the visible result of contraction or may be portions of a very fine and practically invisible meshwork of contractile elements, comparable to the network of myocyte fibrillæ of Gregarines, which are often more or less circular in many of these Sporozoa; but in the Selenidiidæ the fibrils are longitudinal and beaded during contraction, and are, indeed, thread-like myonemes. Further, Perrin stated that the periplast contained fibrils, judging

from macerated specimens. I have not met with macerated specimens myself on which I should care to base any very definite inferences, for in macerated preparations the fibrils might equally well be those of the membrane. However, in addition to what has been stated in the preceding, we have better evidence, I think, from stained normal specimens that contractile fibrils do occur in the periplast, although they are invisible in fresh specimens, and are only seen in some favourably stained preparations, and they are probably comparable to the myonemes of the ectoplasm of Trypanosomes, seen best in Piscine forms (e. g. Trypanosoma rajæ).

In the favourably stained specimens of S. balbianii here referred to, which had been treated with gentian-violet or iron-hæmatoxylin, longitudinal lines or ridges could be seen near the outer edge of the periplast (Pl. 1, figs. 2, 11, *my.*), running nearly parallel to the contour of the body, and better seen in some parts of the organism than others. These lines were quite distinct from the membrane or any part or fold of it (see fig. 2, where the chromatic border of the membrane is evident and quite separate). These are, I think, myoneme fibrils, staining rather deeply violet with gentian dye. Similar appearances may be seen in other preparations.

There are, undoubtedly, myoneme fibrils also present in the membrane, which is an extension of the periplast.

The periplast is, then, hyaline, and consists of viscid protoplasm of a firm texture, which gives a definite contour to the body, and yet is itself flexible. The periplast is directly continued into a laterally extended, spirally arranged, "undulating" membrane. I would prefer to deal with the membrane separately, but at this point it is convenient to consider a recent note by Swellengrebel (**10**). Herein the membrane of a Spirochæte is considered to consist of two distinct parts: (i) a periplastic appendix, and (ii) a chromatic band or ribbon running along the cell, following the appendix, and sometimes beginning in a granule ("centrosome," Pl. 3, fig. 22; also Pl. 1, fig. 5*a*).

Regarding these remarks of Swellengrebel, it seems to me

that he rather emphasises the distinction between periplastic appendix and chromatic border. Further, he does not mention the contractile fibrils actually found in the "periplast appendix," which fibrils appear to stain like chromatin, for they are plainly seen with gentian-violet and iron-hæmatoxylin. After staining normal specimens with the Giemsa and Leishman colorations these fibres are not plainly seen, and that probably accounts for their being overlooked by Perrin. But in the case of disintegrated specimens similarly stained these fibrils can be seen, after patient observation, as faintly pink staining threads (Pl. 1, fig. 10).

I have tried the effect of various reagents on the periplast, but will postpone any discussion of the results to a later and separate section.

Regarding the **endoplasm**, which is not easily differentiated from the other structures, I can only add that it does not appear to contain any vacuoles or enclosures, at any rate in normal specimens. It stains blue with Giemsa's coloration.

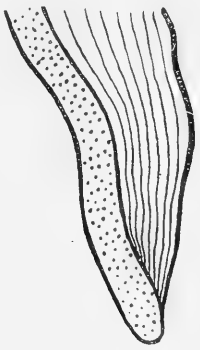
The Membrane.

This characteristic structure of the genus Spirochæta has been already discussed in brief, and the following description is given in addition or extension :—

It is a spirally-wound lateral extension of the periplast with a thickened border, and is composed of almost longitudinally arranged fibrillæ running nearly parallel to the chromatin border (see Pl. 1, fig. 5; Pl. 2, fig. 27). Though nearly longitudinal in disposition, these fibrils probably run with a slight obliquity, as diagrammatically represented in text-fig. 5.[1] These fibrillæ are contractile, and probably contract one after the other in very rapid succession. They may be termed "myoneme fibrillæ," though the term

[1] Although the myoneme fibrils may sometimes appear rather more oblique in some of the figures (e. g. Pl. 1, fig. 3, lower end), this additional obliquity is due to the spiral winding of the membrane, and to its frequent folding (Pl. 1, figs. 2, 5*a*; Pl. 3, fig. 20).

"myoneme," meaning "muscle-thread," is perhaps not an ideal one, yet is far preferable to the term "myocyte" or "muscle-cell" applied to the layer of similar fibrillæ in Gregarines, which are themselves, as a whole, single cells. There appear to be some eight or nine principal fibrils, and many less evident ones parallel therewith, all of them apparently almost longitudinally arranged. Though the existence of a network of fine fibrils is not excluded in this clear, delicate, and narrow structure, such finer fibrils are invisible.

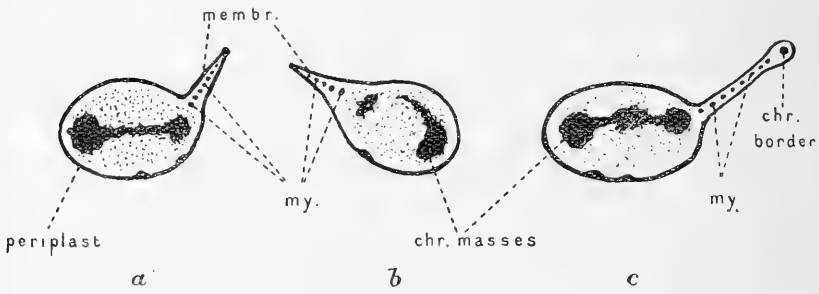


TEXT-FIG. 5.—Diagram to illustrate the probable successive origin near the ends of the body of the myonemes in the membrane, thus giving rise to approximately longitudinal striations in the membrane. The exact origin of these structures is too fine and delicate to determine more definitely under the microscope. No obvious connection of the points of origin of the myonemes with the chromatin rodlets can be seen. A scheme for the possible formation of myonemes is put forward in Appendix III.

In slowly moving specimens of S. balbianii, the edge of the membrane, and even the membrane itself, is easily seen in life under a Zeiss DD objective and oculars 2 or 4. I have been able sometimes to see the edge of the membrane in life even under lower powers. In the case of S. anodontæ it is seen with greater difficulty, but it shows in life even in this case. Such slowly moving forms are curved into about two or three sinuosities only, and the membrane can be seen, in the troughs of these, loosely arranged and not contracted close to the body.

The structure of the membrane herein described is borne out by an examination of transverse sections of an infected crystalline style, as shown in text-fig. 6, and proves it to be an outgrowth of the periplast.

It has been suggested by Laveran and Mesnil (5) that this structure is not really a membrane, but a sheath ("gaine") surrounding the organism, and only attached at the extremities. In their monograph on Trypanosomes Laveran and Mesnil thus set forth their views on this structure:—"Une gaine lâche unie au corps à ses deux

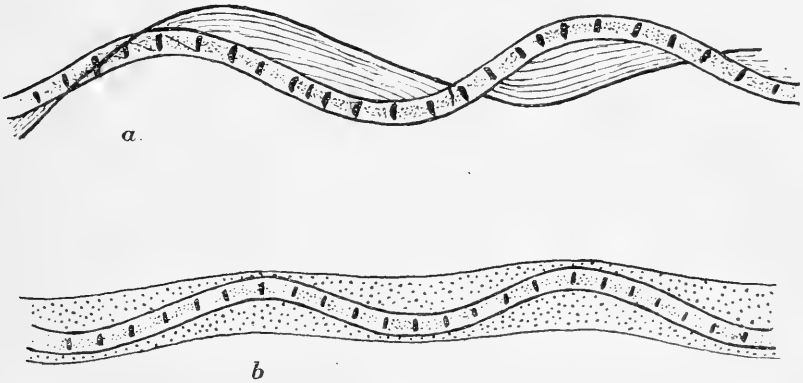


TEXT-FIG. 6.—Diagrams of transverse sections of Spirochætes as seen on sectionising an infected Lamellibranch crystalline style. The membrane is a lateral extension of the periplast containing myonemes (*my.*) and spirally wound. *c* passes approximately through a node of the membrane and shows the thickened chromatic border.

extrémités et dans laquelle ce corps flotte; en tout cas, il n'y a pas de bordure épaissie comme à la membrane ondulante des Trypanosomes." This question is a most difficult one, and is certainly not to be passed over lightly by mere dogmatic assertion either one way or the other. However, one can see in preparations the spiral arrangement of the membrane in most cases and the actual crossing over the body from one side to the other, both above and below (Pl. 3, fig. 22; Pl. 2, fig. 29), and, furthermore, the organism possesses a distinct spiral motion, evidently guided by the spiral arrangement of the membrane. Again, if the body moved more or less freely inside a sheath one might expect

to see some signs of differential motion between the organism and its sheath. I have never seen such during my investigations (text-fig. 7). There is also the question of the longitudinal division of the membrane.

Regarding Laveran and Mesnil's statement as to the non-existence of the chromatic border to this membrane, I am sorry to have to state that I think they are wrong here, and in support of my views I need only refer to the researches of Perrin (**9**) and Swellengrebel (**10**), and to the fact that all



TEXT-FIG. 7.—*a* shows the border of the spirally wound membrane crossing above and below the body as actually seen in both living and stained preparations of Spirochætes. *b*. Sheath ("gaine," Lav. et Mesn.).

research-workers to whom I showed my preparations agreed as to the existence of this thick border (cf. Pl. 1, fig. 5; Pl. 3, figs. 18, 20, 36).

This structure is then a membrane, and is a spirally-wound outgrowth of the periplast. It is composed of myoneme fibrils, and is contractile, and is the locomotor agent of the organism. Its own vibrations are very slight—it is not markedly an "undulating" membrane—and any such slight movements of this membrane are independent of those of the body.

It has been recently suggested by Vlès (**11**) that there is

a ciliate stage in the life-history of S. balbianii—in other words, that the membrane is built up from the agglutination of cilia ("flagella" of English bacteriologists), or even the membrane may be decomposed into these flagella (see figs. 9 and 10). I made some of these preparations myself with Vlès at Roscoff, and have no hesitation whatever in confirming the correctness of the appearances figured by him in his note (**11**). At the time I was favourably disposed, in common with several French protistologists present, towards the interpretation set forward in the note (**11**), but now, after careful consideration and the examination of much more living material, I think these apparent flagella are myoneme fibrils (Pl. 1, figs. 9, 10). They stain deeply with gentian-violet and moderately deeply with iron-hæmatoxylin, but faintly pink with Giemsa's or Leishman's stain. It seems to me that the myoneme fibrils are split off the membrane during its rupture, which sometimes occurs during the violent contortions and death struggles of the organisms, especially in a damp atmosphere like that of Roscoff. The so-called "cilia" then are the frayed out ends of myoneme fibrils, still partially attached to the body of the organism.[1] It may be that the membrane, as recently suggested to me in a private communication from France, is really a "ciliated membrane." This seems to agree closely with the view I had elaborated, and is supported by the peristome membranes of Ciliates. I have never seen these flagella during life, nor have the French investigators judging by their published notes. It is probable that the "ciliate" appearances described by Borrel in the case of S. gallinarum are of this nature, and the principal discrepancy between the accounts of this organism by Prowazek and himself would thus be explained. The fibrillar nature of the membrane is none too well shown by the various modifications of the

[1] Bütschli ('Archiv f. Protistenkunde,' I [1902]) maintains that in Ophidomonas jenensis a terminal flagellum splits into a tuft of finer flagella or filaments. This is a somewhat similar circumstance, and helps to confirm my view.

Romanowsky stain. The principal fibrils do not easily stain deeply, nor do they "stand out" by this method of coloration. Gentian-violet and iron hæmatoxylin give better results. That these fibrils are strictly such and not merely folds is, I think, clearly proved by the occurrence, under certain conditions, of specimens described by Vlès (**11**), and the rupturing of the membrane in dying forms can actually be watched under the microscope. Further, the membrane in specimens of S. balbianii from English oysters is rather too narrow to allow of much folding; far too narrow, I am sure, to allow of the appearance of some eight parallel myonemes by mere folding. Also Keysselitz (**4**) shows, in his figure 13 *a*, the existence of myonemes in a "macerated" specimen of

Text-fig. 8.—This shows myoneme fibrils of the membrane "condensed" at the sides of the troughs formed by the membrane with the wavy outline of the body. These are not due to folds.

S. anodontæ. I think it is quite likely that his specimen was not so much "macerated" as that in dying the membrane of the parasite had relaxed. However, Keysselitz in his figure clearly shows the presence of myoneme threads, not "folds." And again, the fibrils are condensed at the sides of the troughs, formed by the membrane and wavy body, where the membrane is seen passing over the side of the body (text-fig. 8). I do not understand myself how these "condensation" appearances could be explained by the edges of folds.

In life, during active movements, the membrane is closely contracted round the body, and is not easily seen except as a halo around the organism. In faintly stained fixed preparations, where the membrane has not properly taken up the stain, similar appearances occur.

The membrane[1] of S. anodontæ (Pl. 2, figs. 27, 29, 30, 31, 32; Pl. 3, fig. 39) is most difficult to discern and to stain, more so than that of S. balbianii. In both cases, a membrane is undoubtedly present, sometimes very narrow in very attenuated forms (cf. Pl. 3, fig. 23). The structure of the membrane of S. anodontæ conforms to that described for S. balbianii, namely, a matrix of hyaline, viscid protoplasm directly continuous with the periplast of the body, and strengthened by a number of almost longitudinally arranged myoneme threads (Pl. 2, figs. 27, 29).

The chromatin border of the membrane is stated by Perrin (**9**) "to be continued into a fine line ending in a dot, which is connected with the nuclear band" at one end. Swellengrebel (**10**) mentions that this border sometimes commences in a granule. This is very difficult to observe. I have sometimes seen such a dot, but only at one end, not at each end, as one might expect from the ease and frequency with which these organisms suddenly reverse their direction of motion. Such a dot may be termed a "centrosome" (Pl. 3, fig. 22).

Closely connected with this in S. balbianii is the occurrence at the extremity of each of the rounded ends of a larger dot or granule of chromatin (Pl. 1, figs. 5, 5*a*; Pl. 2, fig. 27), "the thickened nodules of periplast" of Perrin (**9**). Each of these dots divides into two smaller dots, lying side by side, in parasites about to divide (Pl. 3, figs. 19, 40). These dots stain red with Giemsa's solution, and are deeply coloured by gentian-violet or iron-hæmatoxylin. They are, I think, composed of chromatin and are "basal granules" comparable with those occurring in Trypanosomes, as described by Schaudinn (**20**) for T. noctuæ.

The ends of S. anodontæ are tapering, not rounded as in S. balbianii, and each tapering end appears, with Giemsa's stain, as a red thread, about 4 μ long (Pl. 3, fig. 37). At the

[1] Through the kindness of Professor G. H. F. Nuttall I have been enabled to examine a stained preparation of S. duttoni. I believe it possesses a narrow membrane, which has not been recorded before, but I should like further material to make quite certain.

base of each terminal process or filament in favourably stained specimens a small red dot or fine granule is seen (Pl. 3, fig. 38, *b.g.*). These are the "basal granules." Strictly I think the ends of S. anodontæ are quite alike and that each one is pointed,[1] especially after looking at living specimens. But in some stained preparations one is apt to get specimens with one end a little more rounded than the other (Pl. 2, fig. 34), such differences possibly result during the making of the stained preparation, and are probably neither natural nor normal.

The significance of the terminal processes of S. anodontæ is interesting. They seem to be stiff. The membrane is probably directly continuous with them, at any rate the processes are directly continuous with the periplast, indeed, are a part of it. Are they chromatin-containing flagella, the fine prolongations of the chromatin body of the membrane? This is a difficult question when dealing with such minute and fine structures. I prefer to leave the question still open, only expressing an opinion that they do not appear to be motile flagella, and that S. anodontæ is strictly a Spirochæte, possessing pointed ends.

The ends or membrane of S. balbianii are not thus prolonged. In this species the membrane does not extend quite to the ends of the body by a distance of 2μ to 3μ. S. plicatilis also possesses rounded ends.

No flagella could be detected in the case of S. balbianii, even after the use of appropriate reagents, such as tannin.

It may be of interest to mention that specimens of Trypanosoma gambiense, stained with gentian-violet, did not exhibit any fibrils in the membrane, nor markedly on the surface of the body. Gentian-violet, indeed, was not a successful stain for showing the structure of T. gambiense—it was not precise, but vague and diffuse. The appearances of the membrane, after staining with gentian, were quite different in the cases of S. balbianii and T. gambiense. It might, even, be not too wild to consider whether or not this

[1] In S. duttoni one end is said to be pointed while the other is rounded (12).

difference in the reaction of the two organisms to gentian-violet is not worthy of account as a possible factor in discussing the question of the affinities of the two organisms.

It has been suggested by Minchin (**17**) that an undulating membrane is of value to a Protist possessing it as a means of pushing aside obstacles such as blood-corpuscles occurring in the medium in which it lives. This may be so, but it seems to me that the primary function of the membrane of Spirochætes is that of a locomotor agent. Also, few obstacles are encountered by Spirochætes while they remain within the style itself. However, Minchin's suggestion is an interesting one, for much débris and partially digested food are encountered by S. balbianii and S. anodontæ while they are free in the lumen of the gut.

The Nucleus.

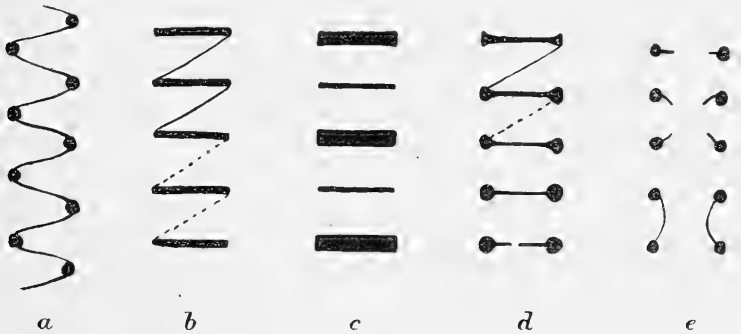
The nucleus of these two Spirochætes under discussion is diffuse, and is disposed over the whole length of the attenuate cylindrical body, except just at the extremities, about 2 μ from each end.

In the more commonly occurring forms of S. balbianii, those presumably in the resting state, the nucleus consists of transversely arranged bars or rodlets of chromatin disposed at nearly regular intervals, and placed transversely to the long axis of the body. These chromatin masses appear as rodlets, but it is possible that they might be discs or bands. To decide what is the real character of the chromatin masses is not at all easy in such a small organism, S. balbianii being a cylinder of 2 μ diameter, and S. anodontæ only about one third of that diameter—namely, about 0·7 μ.

I think, however, that these masses are rodlets, because on focussing they are not seen passing through to the far side, as would occur in the case of peripheral bands. If they were discs, that is, continuous right across the interior of the organism, they would divide the organism into segments, and the parasites might be expected to separate easily at, or

between, these points as nodes. Such transverse division into many segments does not occur, and the organism is a unicellular Protist. But one can usually focus down to these bars, and then below through a granular endoplasm, in favourably stained specimens under the best conditions of illumination. Also, the rod-like character of the chromatin is shown in transverse sections (vide text-fig. 6).

Again, there is, undoubtedly, a spiral, achromatic, connecting thread more or less evident at different periods of the life of the organism, which seems to me to be not easily



Text-fig. 9.—This shows the nuclear helix in Spirochætes, and its various forms and correlations: *a*. Loose helix, with chromatin masses at the turns, connected together chiefly by achromatic substance. This stage is seen during the activity of the organism. *b* shows scalariform rodlets with slight amount of achromatic substance. *c* shows rodlets, some thicker than others because they were originally arranged on a helix, and so were at different depths of focus. *d* shows division of the rodlets into dumb-bell shaped chromatin masses. *e* shows longitudinal division of the organism. The halves of the dumb-bell shaped chromatin masses are contracting to the periphery—the remains of the threads are "looping-up."

compatible with the idea of discs (Pl. 1, figs. 5, 11; Pl. 2, figs. 13, 15, 16, 27).

Returning now to the rodlets seen in the normal form, if one looks carefully at such rodlets one finds certain irregularities as depicted in text-fig. 9, such as some bars being thicker than others, some thicker at one end and yet others broken into dots.

In other forms the transverse rodlets are united by faintly

staining zig-zag connections (Pl. 1, fig. 3) not very evident and so probably not always seen in the first-mentioned forms. However, these irregularities can be explained by correlation with yet a third set of forms, already mentioned, wherein there is a spiral thread in which granules of chromatin are distinctly seen (Pl. 1, fig. 5; Pl. 2, fig. 27). These various forms of the nucleus are illustrated in text-fig. 9.

It seems, then, that the nucleus of these Spirochætes really consists of a spiral achromatic portion on which granules of chromatin are arranged. The question next to be considered, is the chromatic filament a spiral flattened into a zig-zag in the median longitudinal plane, forming a core to the organism, and passing down the centre of the body, or is it a marked helix (comparable to the solenoid of the physicist), with the coils of the helix coming near the surface of the body, that is, almost touching the periplast? The latter alternative is, I think, the correct one—namely, that the rodlets or granules are arranged on a helix rather more loosely coiled at some periods in the life of the parasite than at others.

Analogous conditions of the nucleus have been briefly described by Schaudinn (**21, 34**) in the case of Spirochæta plicatilis, where the diffuse nucleus consists of a spiral thread on which occur masses of chromatin at regular intervals. The spiral filament, judging from Schaudinn's figures is not entirely achromatic, but may contain some chromatin in the form of chromidia disposed along it.

I have myself examined some of the Spirillar forms occurring in the hind gut of the Cockroach. There are examples, apparently, of at least two genera, Spirillum and Vibrio. However, I have not gone into the question of the systematic position of the Bacteria occurring there, but rather an examination of the condition of the nucleus of such forms. After fixation of wet films with osmic vapour, also with absolute alcohol, and staining with gentian-violet, which revealed the presence of terminal flagella in the Spirillum, a diffuse nucleus was observed (Pl. 2, figs. 24—26), consisting of a

number of chromatin masses seen to be connected by a lightly staining spiral in successfully stained specimens. At other times the achromatic thread was not evident. The condition of the nucleus was that seen in Spirochætes on a smaller scale, for these organisms are minute. Swellengrebel (**10**) has described such a structure for Spirillum giganteum.

Further, Schaudinn (**20a**) has also described the condition of the nucleus in Bacillus bütschlii, where the nucleus in the resting condition consists of free chromidia scattered in the protoplasmic network, which chromidia, in preparation for spore formation, condense at the poles with a spiral thread of chromatin connecting these poles longitudinally, and along this spiral thread chromatin dots or chromidia are discernible.

The more marked or obvious condition of the spiral is, I think, probably connected with some period of relatively marked activity in the life-history of the organism, while the scalariform arrangement of transverse bars or rodlets with less evident connections of achromatic substance is, it seems to me, typical of the trophic condition of the organism.

The structural inter-relations of these two forms or conditions of the nucleus in Spirochæta balbianii apparently arise thus. The transverse bars or rodlets are each composed of two chromatin masses (or groups of chromidia) which have joined along the helix, the chromatin substance of each of the constituent chromatin masses being uniformly disposed along the rodlet thus formed. In this process the core of the achromatic filament is somewhat altered—it is flattened—and the chromatin is concentrated in the rods.

In the case of division—which is usually fission in a longitudinal plane, that is, along the principal or long axis of the organism—the chromatin rodlets become dumb-bell shaped by contracting in or constricting across the middle (text-fig. 9, *d.*). The chromatin substance flows into and concentrates within the heads of the dumb-bells arranged along the periphery of the body, and then the dumb-bell shaped rodlets break across the centre. This condition would result from

an attempt at loosening the spiral or coil of the helix during a period of activity such as occurs at division. It has already been mentioned that a slightly loosened condition of the spiral is probably to be correlated with a period of activity. The resulting chromatin masses, after the division of the dumb-bell shaped rodlets, do not seem to be arranged always exactly opposite each other along the periphery of the body, owing to the slight loosening of the spiral just mentioned, and to the general torsion of the body. In the daughter forms thus produced, the chromatin masses and the remains of their attached threads join up (text-fig. 9, *e.*), and so form a very slightly coiled spiral or even a longitudinal rod, such as one sees in long attenuate forms of the parasite, which are probably young forms, though some protistologists would consider these to be male forms. But the condition of the nucleus as a central longitudinal rod may result from over-staining—which is often necessary for showing the details of the membrane—when the true spiral nature is obscured, and this must be borne in mind in interpreting the condition of the nucleus of these Spirochætes.

The above statements represent the views I have formed, as a result of observation, on the nucleus of S. balbianii. I am sorry I do not agree in all details with Perrin (**9**), but I am unable to correlate some of his complicated phases with the appearances of the actual specimens. However, I have the greatest admiration for Perrin's work, which was largely that of a pioneer in elucidating the nuclear details of S. balbianii.

Swellengrebel (**10**), in his recent note on Spirillum giganteum, mentions the arching or vaulting of the chromatin filament (. . . "le filament former une anse"), and thus supports the view of the helicoid nature of the spiral, for he states that the same nuclear structure obtains in S. balbianii. This disposes of the ideas of the earlier observers, especially Lustrac (**6**), who considered the centre of the organism to be alveolated, and overlooked altogether the presence of chromatin, but that was only to be expected

at the time with the appliances then available for such research.

The nucleus in S. anodontæ is much the same as that of S. balbianii, though the organism is considerably smaller, and the details are consequently much more difficult to discern. The organism also moves much more quickly, and there may be the added torsion of the body, in fixed and stained preparations, in the case of this smaller parasite. Nevertheless, we find the nucleus disposed over the general length of the body, and not marked irregularities present in the distribution of the chromatin, as figured by Keysselitz (**4**, fig. 13*c*). Such irregularities might be due to abnormal specimens or imperfect staining. Stress has already been laid on the unusual difficulty of differentiation in staining with iron-hæmatoxylin in such minute forms, which are too small for one to observe the details of differentiation under a Zeiss DD lens.

There appears to me to be no evidence to support the queried suggestion of Keysselitz himself (**4**, fig. 13*d*) as to the marked contractility of the chromatin thread. I have never seen a condensed condition of the nucleus in any of the specimens of either of the Spirochætes which I have examined, although I have carefully searched for the same. I have not seen, then, in S. balbianii or S. anodontæ a concentrated central condition of the nucleus as figured by Schaudinn for Spirochæta (Trypanosoma) ziemanni (**20**), and which is so common in most Protozoa and the Metazoa.

The achromatic spiral filament, which sometimes contains chromidia disposed along it, is probably homologous with the "karyosome" of higher forms, for it consists of achromatic nuclear substance in which granules of chromatin may occur, as judged by the best chromatin stains, such as iron-hæmatoxylin and gentian-violet. I have hesitated to make direct use of the word "karyosome" in my descriptions of the nucleus, pending the discovery of some further connection between the "diffuse" and the "concentrated" conditions

of the nucleus—which probably exists in other organisms showing morphological characters between Spirochætes and the undoubted Protozoa, and which have yet to be examined.

Similar remarks apply to the use of the word "chromosomes" for the chromatin masses occurring along the spiral filament, and the use of which term by Perrin has been previously referred to.

I have not seen definite evidence of reducing division of the chromatin in these organisms.

Division.

The method of division in Spirochætes is much disputed, some authorities, as Laveran and Mesnil (**5**), maintaining that it is transverse as in Bacteria generally, while others, as Perrin (**9**), maintain that it is longitudinal, while Certes (**1, 2**) and Lustrac (**6**), most correctly, I think, find both methods taking place. Division usually occurs in the crystalline style, especially in the case of S. anodontæ, less frequently in the gut, but I have seen it there in S. balbianii, when no style was present in the oyster.

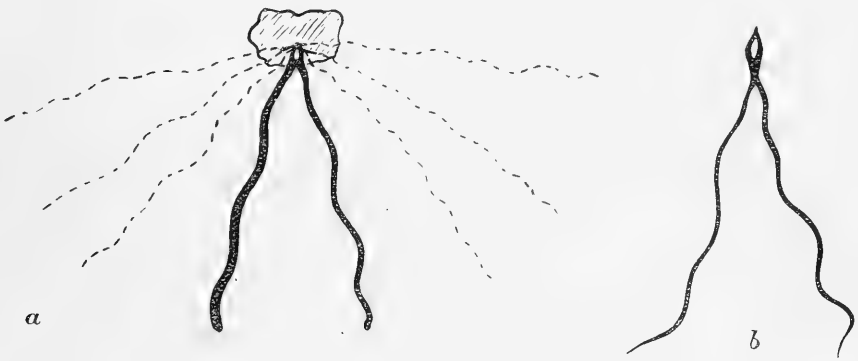
Longitudinal Division.

The usual method is by longitudinal fission, the two daughter individuals resulting from a common parent being very thin and sub-equal.

The division is first apparent in the membrane (Pl. 2, figs. 13 to 16), but this is really preceded by division of the "basal granules" at the ends of the periplast. This feature has not been noted before. Each basal granule early divides into two in forms preparing for division, and the two daughter granules may remain attached in a dumb-bell like manner for a time (Pl. 2, fig. 15; Pl. 3, fig. 40).

I cannot agree with Swellengrebel's ingenious suggestion (**10**) that the chromatic border of a membrane can be divided

into several strips, and thus simulate a longitudinal division by folding of the membrane. In his own words: "Cette bande peut se diviser en plusieurs cordons et donne ainsi l'illusion d'une division longitudinale de la 'membrane ondulante.'" He has probably mistaken the myoneme fibrils in this case. There is no illusion in the matter, for longitudinal division can be observed under the microscope, and so is undoubted. But Swellengrebel only sees one basal granule in S. balbianii, while he maintains that there are two in Spirillum giganteum.



TEXT-FIG. 10.—*a*. S. balbianii; *b*. S. anodontæ, showing longitudinal division. The partially separated forms are gradually increasing the angle of divergence between them. A vacuole is seen in the cytoplasm at the undivided end.

I can confirm in the main the details of longitudinal division as given by Perrin.

The basal granules of each end appear to divide simultaneously, but only those of the one end break contact and definitely separate, so that the daughter individuals resulting from fission remain attached at one end often for a long time. Occasionally, however, I have noticed forms attached by each end, as figured by Lustrac (**6**, Pl. 1, fig. 1). I think such were true cases of longitudinal division, and not mere intertwining of two forms lying side by side, but otherwise unrelated.

In the usual method of longitudinal division the parts gradually separate (Pl. 2, fig. 17) by wriggling till they are finally 180° apart, but they frequently remain for a long time attached at one end with the free ends diverging about 30° to 40°, like the legs of a compass (Pl. 3, fig. 18), and executing very rapid movements the while. The unseparated end is often fastened to débris (text-fig. 10, and Pl. 2, fig. 28), and the dividing forms remain in the same place during the process.

On two occasions I watched the partially divided forms wriggling actively for about an hour, not always in unison, but usually so. It seems almost impossible to follow the whole process in life, for on each of the occasions that I had the opportunity the forms died before the completion of the division. Perrin also mentions this difficulty, for he watched a pair for forty minutes, and these died before the completion of division.

The division of the chromatin masses takes place early, in the manner described in the preceding section when discussing the nucleus and the significance of its various forms.

Judging from stained preparations the order in division probably is: first the basal granules, next the membrane, then the chromatin masses, but the interval between the stages is often slight.

The nearly separated organisms remain attached only by the still unsplit end of the periplast of the parent, a vacuole appearing in the common portion of the periplast just before final separation.

Regarding some of the phases of nuclear division mentioned by Perrin (**8**, **9**), I was unable to trace his division of a karyosome rod into bacilliform and dumb-bell shaped segments, followed by transverse and then longitudinal division.

I have seen longitudinal division in life in S. anodontæ also, and this is the usual direction of fission in the Spirochætes of Anodon (Pl. 3, fig. 40).

Transverse Division.

This has been stated to occur by Certes (**2**), Lustrac (**6**), Laveran and Mesnil (**5**), and Swellengrebel (**10**). It was emphasised as the only method by Laveran and Mesnil, but Perrin ingeniously suggested that this was to be explained as longitudinally divided forms, separated by 180°, and not yet finally disconnected. It seems to me that the determination of the number of chromatin masses ("chromosomes" of Perrin) might shed light on the matter, and probably settle the point.

TEXT-FIG. 11.—Outline of a specimen of S. balbianii vibrating about a non-central node. It did not divide.

But these are not always easy to see separately or to count in stained preparations. For example, the number of well-marked chromatin masses was thirty-eight and forty-three respectively in the case of transversely dividing forms mentioned below (see Pl. 3, fig. 19). Some of the thinner bars of chromatin may have been added by transverse division of previously thicker single ones, but the question of torsion must always be borne in mind.

Personally, I believe that transverse division[1] does occur in addition to longitudinal but less frequently, for, in the case of S. balbianii, both long and short forms are seen.

[1] See Note on page 68.

In some stained preparations there were somewhat long forms with the membrane discontinuous in the centre, where a vacuole-like space occurred; the edges of this space were sharp, not torn, while the periplast appeared just continuous over the gap (Pl. 3, fig. 19).

Further, in living specimens one sometimes sees forms vibrating about a node, possibly not central, but I have never seen division about such a point actually occur during life (text-fig. 11).

It is interesting to add regarding the form shown in Pl. 3, fig. 19, that each basal granule, at either end of the transversely dividing form, was—in both cases—itself dividing into two, while the whole nucleus showed signs of approaching division; so that it was likely that the daughter forms, themselves produced by transverse division, were in each case about to divide immediately by longitudinal division.

General Note on Division.

I should like to add the following remarks, from the physiological point of view, to my discussion of the phases of the nucleus during rest and activity respectively, given in the preceding section.

Normal protoplasm is always in a state of slight contraction known as "tonic contraction"—which, applied to muscles, is the so-called "muscular tone."

Owing to the resistance to the movements of the organism by the fluid in which it lives, the migration of protoplasmic particles towards the periphery occurs in accordance with the physical laws regulating the motion of fluid particles. Under such conditions the contraction is increased, and in the case of fission resulting it would be rendered more easy because under these conditions of increased contraction there would be less fluidity in the protoplasm at the periphery of the organism than at the centre.

Concentration of the protoplasm at the edge would further result from the "tone" being increased, and so, with the

concomitant nuclear changes already noted, longitudinal fission of the organism would result. Electrical charges induced by the movements of the chromidia would bring about such increase of "tone."

Attempts at loosening the helicoid spiral of chromatin of a Spirochæte, as during such a period of activity as division, would cause the bars or rodlets of the resting phase to become dumb-bell shaped, that is, the chromidia would concentrate at the periphery and the rodlets, after becoming dumb-bell shaped, would ultimately break across the centre.

If, then, the flow of protoplasm is towards the periphery, that is, in the direction of the path of the chromidia and away from the centre of the organism, fission would be rendered still more easy. (Witness slight thickening of myonemes, shown rather faintly [Pl. 1, fig. 2].)

Note on Polymorphism, Conjugation, Encystment.

It is necessary, I think, to consider these matters, more especially from the point of view of completeness, for there is little evidence, at any rate at present, to support the existence of these processes in Spirochætes.

Sexual Polymorphism.—Perrin (**8**, **9**) in his account of S. balbianii differentiates between "male," "female," and "indifferent" forms on certain morphological grounds, but "chiefly by their behaviour under adverse conditions" (**9**, p. 135). This basis of differentiation is, it seems to me, unsound, for under such conditions one is probably dealing with abnormal forms. I fear too much systematic work already, perhaps, has been based on pathological specimens.

Perrin himself states (**9**, p. 137) that:—"The morphological differences between the two forms [indifferent and female] are thus small, and, unless . . . . the features [largeness and stoutness] are sharply marked, it is by no means always easy to tell whether a given individual belongs to the female or to the indifferent type."

The "working hypothesis" of sexual polymorphism, as set

forward by Schaudinn, is excellent, but it is merely an "hypothesis," and Schaudinn himself only put it forward as such (**34**), and did not unduly force it beyond this stage. This conception of polymorphism in relation to sex may be applied perhaps to Spirochætes, as it is to Trypanosomes, thus allowing of the possible existence of male, female, and indifferent forms, the former ("male") being characterised in extreme cases by the possession of a thin elongate body and clear protoplasm, the "female" being stouter and larger, with granular protoplasm containing reserve material.

Returning now to the so-called forms in S. balbianii, Perrin mentions the elimination of chromatin at a swelling in the centre of the Spirochætes, comparing this to a "maturation" process or "reducing division" of his "chromosomes." But it is possible that this was really due to bursting of the periplast and outflow of some of the endoplasm and nuclear material.

Of the many hundreds of specimens which I have observed of both S. balbianii and S. anodontæ, I find no evidence whatever for definitely stating the existence of polymorphism in relation to sex in any of these Spirochætes. I do not mean by this statement that sexual forms do not exist in some possible portion of the life-cycle at present unknown, but I think there is little evidence as yet in this direction, in spite of careful searching and consideration. It is useless, at this stage in our limited knowledge of the Spirochætes, to force matters farther. Schaudinn's "working hypothesis" on this question, as such, is excellent, but the hypothesis is far from being proved, and so is not a "theory." I think the differences of appearance between Perrin's male, female, and indifferent forms are so slight and so gradual, that they are probably only the extremes from a more or less continuous series. Recently Moore and Breinl in a note ('Lancet,' i, 1907, pp. 1219–20) on male and female forms in Trypanosoma gambiense support this "series" view.

I am convinced that no useful purpose is served by further discussion at this juncture. And assuming that the Spiro-

chætes are Bacteria, which is quite possible, little progress is made in the idea of "sexual polymorphism" from that standpoint.

Conjugation.—Perrin mentions this as occurring very rarely. He says it "appears to take place when the crystalline style melts in the gut of the oyster." Little is known of conjugation in the Flagellata in general, and I have not seen it in these Spirochætes.

Encystment.—I am sorry that I have no evidence of this in any of my investigations, although the conditions pointed out by Perrin, such as starving the oysters or watching the forms at the periphery of the style, were carefully noted.

I think that Mesnil in his review of this question of encystment, as set forth by Perrin, was probably justified in regarding such forms—together with the formation of "male gametes"—as representing really "involution forms," resulting from unusual or unfavourable environment.

Further, Swellengrebel (**10**) accounts for the so-called encystment of S. balbianii thus, in his own words: "Quelquefois il se forme des boules plasmatiques, de structure alvéolaire homologue à celles des Spirilles. Ce sont là vraisemblablement les 'kystes' de Perrin."

Environmental Effects. Cultures. Chemical Reactions.

Effects of Temperature.

At the temperature of the laboratory, 15° C. to 20° C., the Spirochætes live in their natural medium from a few hours up to 48 hours. At 30° C. the Spirochætes died in about half an hour or less. At low temperatures, as 5° C., the Spirochætes lived four or five days.

Effects of Alteration of Osmotic Pressure.

In distilled water S. balbianii died very soon, though exceptions to this occurred. Even in the case of such exceptions the membrane ultimately macerated and death resulted.

The periplast swelled slightly but did not burst for a long time. This Spirochæte lived a little longer in tap-water, which was slightly alkaline. S. anodontæ lived for a short time, up to one hour, in sea-water. In white of egg S. balbianii and S. anodontæ died very quickly.

Both organisms lived for a time in physiological salt solution (0·75 per cent. sodium chloride), though S. balbianii did not seem "happy" therein.

Plasmolysis.

This really should come under the preceding heading, but is well considered separately. I agree with Swellengrebel that these organisms are implasmolysable. In 4 per cent. salt solution S. balbianii lived for half an hour; at first its movements were active, but slowed down afterwards. S. anodontæ also died in this strong solution. The periplast, however, did not swell up, and there was no bursting so far as could be perceived by Zeiss DD and ocular 4. The organisms therefore appear implasmolysable.

Oysters infected with Spirochætes have been kept for a fortnight in constantly changed sea-water, as previously mentioned. Specimens of Anodonta cygnea in which Spirochætes occurred were kept in running water for over a month and were still infected, though apparently the infection declined in intensity, while others kept in an aquarium for about two months were found to be still infected.

Aërobic Reaction.

These Spirochætes did not appear to mass themselves especially round air-bubbles.

Cultures.

Many attempts were made at preparing artificial media for the Spirochætes under discussion, but all failed to sustain the life of the organism for any length of time.

Perrin's mixture of egg-albumen (one part) and sea-water (two parts) was found to be useful, even when no special care was taken to concentrate the constituent sea-water to two thirds of its bulk, in order to make the mixture isotonic with ordinary sea-water. In this the Spirochætes remained alive for several days, though their movements gradually slackened.

A weak extract of the liver of the oyster in sea-water—made by grinding together a little of the tissue of the organ and sea-water with carborundum powder and rapidly filtering, and to which excess of sea-water was added, the whole being made sterile as far as possible—was found to be effective, and the organism (S. balbianii) lived therein for at least twenty-four hours, sometimes even nearly forty-eight hours. It is difficult to maintain the sterility of the various artificial media tried, almost impossible in fact, for extraneous organisms are of necessity introduced with the gut contents of the Lamellibranch.

A bolder experiment, namely, that of making a weak solution of the crystalline style of Anodonta cygnea in sea-water and placing some living specimens of S. balbianii (from the oyster) therein, was tried. The Spirochætes lived for about an hour in the medium.

S. anodontæ lived for some days in an extract of the liver of the Anodon prepared in the water in which the Lamellibranchs lived. It also lived well in a preparation of the gut-contents of the host, up to the second day after the death of the host.

It may be mentioned for the sake of completeness that some bacteriological media were tried. Among such media were broth, agar, blood-serum, and a mixture of egg-albumen and glycerine; but all were found to be useless in sustaining the life of the Spirochætes therein.

Novy (18) mentions that he was unable to prepare a useful artificial medium for S. obermeieri of relapsing fever.

The inability to find suitable artificial media for keeping Spirochætes alive is an argument in favour of their Proto-

zoal nature as contrasted with their possible Bacterial nature.

Chemical Reactions.

Many attempts were made to determine the chemical nature of the periplast of these organisms. Since Spirochætes may be either Bacteria or Protozoa, special tests were applied for "fungus-cellulose." At the outset it must be mentioned that the nature of "fungus-cellulose" is but very vaguely understood, for after consulting works of reference (**30**, **31**) on the subject, as well as seeking advice from botanical experts, it was still found that the so-called tests for this substance were themselves ill-defined.

Iodine solution stains the organism brown.

Iodine and concentrated sulphuric acid coloured the organisms light yellow, not blue; thus cellulose was absent, but fungus-cellulose might be present. Some retained their shape under this treatment for quite a long time, and the nuclear core and even the dots along it could be focussed, so that these dots were probably not mere optical effects.

Potash in 10 per cent. solution, when applied to these organisms, showed striations in the periplast. Some of the organisms retained their shape for a long period, and were not dissolved. Insolubility in alkalies is characteristic of chitin, and fungus-cellulose may be absent.

Concentrated sulphuric acid alone dissolved the outer portion of the organism (the periplast) after about three hours, but the membrane was not especially soon dissolved. The nuclear core remained undissolved at the end of this period. There was no obvious charring. These reactions with strong acids again rather point to the presence of a quantity of a chitinoid substance, and not of cellulose.

Potash solution was applied to some of these organisms, and afterwards iodine and sulphuric acid. There was no blue coloration evident, and as fungus-cellulose should appear blue after these reagents when applied in the order

named, it is apparently absent—but this test is uncertain in its results. Fungus-cellulose then may be absent.

Bleu-de-Lyon stained the organisms blue, but only faintly. Callose—a supposed constituent of fungus-cellulose—should stain intensely blue under these circumstances. Fungus-cellulose then does not seem present.

Strong acetic acid when applied to the Spirochætes did not dissolve them; on the contrary, they maintained their shape for a long time, though sometimes a certain amount of fraying occurred.

The Spirochætes gave the Xanthoproteic reaction, namely, a yellow or reddish-yellow colour after nitric acid and ammonia. This shows the presence of proteid.

Unfortunately little material could be spared for some of these reactions, and micro-chemical tests are well known to be most difficult on such small organisms. S. balbianii was usually employed, for S. anodontæ was rather too small for these purposes.

Definite conclusions then are most difficult to arrive at. On the whole fungus-cellulose may be absent, and a certain (? small) amount of a chitinoid substance appears to be present in the periplast and the membrane (compare the cuticle of the earthworm).

Affinities and Systematic Position.

Affinities with the Bacteria.

That Spirochætes are Protists is perhaps hardly necessary to state, as doubtless all will agree to this. The question then arises, are they Protozoa or are they Bacteria.

Taking first the principal characteristics that rather suggest their inclusion among the Bacteria we note—

(*a*) The diffuse condition of the nucleus, directly comparable to that of Spirilla, and somewhat like that of Bacillus bütschlii.

(*b*) The occurrence of transverse fission among the Spirochætes, but this is probably not very frequent.

(*c*) The absence of a blepharoplast in the form in which it occurs in a Trypanosome.

Affinities with the Protozoa.

As characteristics favouring their inclusion among the Protozoa we may note—

(*a*) The possession of a membrane.

(*b*) The occurrence of longitudinal fission.

(*c*) They are implasmolysable.

(*d*) Bacteriologists with whom I have conversed attach importance to what they consider the definiteness of the nucleus in these forms compared with the condition of the nucleus (or its seeming absence) in many Bacteria. Undoubtedly chromatin masses occur ("chromosomes" of Perrin) together with an achromatic thread. But there is probably chromatin present in the form of scattered chromidia in all Bacteria.

(*e*) Bacteriologists also comment on the large size of S. balbianii but S. anodontæ is much smaller, while a species like S. obermeieri is smaller still. Little importance is to be attached to size.

(*f*) The presence of a small amount of a probably chitinoid substance in the periplast and membrane. "Chitin" is an animal product, but I do not think it is present to any great extent, and only in a diffuse condition, for the body of Spirochætes is very flexible—and there are probably several kinds of chitin.

(*g*) The inability, up to the present, to find suitable artificial (bacteriological) media in which the Spirochætes may be kept alive.

Longitudinal fission, I believe, is known among the Bacteria in Pasteuria ramosa and Bacillus maximus—also probably in the case of the diphtheria bacillus.

It has been stated (**12**) that S. duttoni is a Protozoon because regular recurrent relapses of tick fever result from its presence in the system. But apparently relapses may result from diseases due to Bacteria, as in typhoid or pneu-

monia. However, I would crave indulgence in discussing these matters, as I am not a medical man.

Then again, Prowazek (**19**) mentions that S. gallinarum may occur for a time inside a red blood-corpuscle of the fowl and that this character of "Zell-parasiten" is against the parasite being a Bacterium. Regarding intra-epithelial stages in S. balbianii or S. anodontæ there is nothing definite, indeed, I do not think that a definite intracellular phase occurs in the life-cycle of these parasites.

On the other hand, Borrel (**14**) in his note on the "cilia" and transverse division in this same species, S. gallinarum, places all Spirochætes in a group of the Bacteria, which he styles the Spirillo-bacteria.

Minchin (**17**), in his recent article on the Protozoa, considers the Spirochætes after the Trypanosomes, as "close allies to the Trypanosomatidæ." Both families possess a membrane, but in the case of Spirochætes the membrane does not markedly undulate as in Trypanosomes, and it is spirally wound. The condition of the nucleus is very different in the two cases; there appears to be no motile flagellum in Spirochætes, and there is a difference in the basal granules in the two cases. These differences were, I think, emphasised by Schaudinn himself when he changed his views on S. ziemanni.

After carefully balancing these characteristics, I think the Spirochætes are quite distinct from the Trypanosomes, showing on the whole, a less highly specialised morphology, and rather exhibiting morphological resemblances to the Bacteria.

A suggested New Class of the Protozoa.

Hereafter it may be necessary to set up a new (fifth) class of the Protozoa for the inclusion of such organisms as the Spirochætes. At one time the name Spirochætacea had occurred to me as a possible one for such a class. This class would possess affinities on one hand with the Trypanosomes,

probably through forms like T. ziemanni, and on the other hand with the Bacteria through forms like the Spirilla in which Swellengrebel (**10**) says there is a periplastic appendix. However, this classification is perhaps a little premature, for probably enough is not yet known of Spirochætes, and other allied organisms which might be included therein, to warrant the institution of such a class at the moment. Considering the present state of our knowledge, then, one must hesitate to push matters further.

The characters which could be cited for distinguishing such a class are:

(*a*) Elongate, thread-like body.

(*b*) The presence of a membrane—the so-called "undulating" membrane.

(*c*) The absence of flagella.

(*d*) The flexibility of the body, which is bounded by somewhat soft (compared with Spirillum), yet definite, periplast. The body is not contractile, since it retains its dimensions, that is, there is a constant ratio between its length and breadth.

(*e*) The ends may be rounded or prolonged into a stiff process. This last characteristic concerning the ends is variable, but in the type species S. plicatilis the ends are rounded.

(*f*) The occurrence of longitudinal division.

(*g*) The presence of a probable chitinoid substance in the periplast.

(*h*) The nucleus consists of a spiral filament, on which are arranged transverse bars or rodlets of deeply staining chromatin.

Schaudinn (**21**), in 1905, discovered the presence of an undulating membrane in the type species, and emphasised this as a characteristic of the genus Spirochæta.

The question of the difference between pointed ends and flagella is rather a fine one, but is decided on the grounds of the stiffness and lack of motility of a process, and the possession of flexibility and motility by a flagellum.

Forms like Treponema pallidum, without membranes, are now not included in the genus Spirochæta, and in Treponema the coils do not result from movement, but are apparently preformed. Similarly, an organism described by Castellani from "yaws" (S. pertenuis) is not a true Spirochæte. However, S. refringens (Schaudinn), a form associated with Treponema pallidum in syphilitic lesions, remains in the genus.

Some of the well-known species of the genus beside S. balbianii and S. anodontæ are:

S. obermeieri (Cohn), from human relapsing fever.
S. anserina (Sakharoff), from the blood of geese.
S. gallinarum (Marchoux and Simond), from the blood of fowls.
S. duttoni (Breinl and Kinghorn), from African tick fever in man.
S. refringens (Schaudinn), associated with Treponema pallidum in syphilitic lesions.
S. buccalis (Cohn).
S. dentium (Koch), from the human mouth.
S. muris (Wenyon) or S. laverani (Breinl and Kinghorn), from the blood of mice.

The accounts of the structure of most of these forms are either incomplete or disputed, and often turn on the presence of a membrane and lack of flagella. There are other doubtful species, and the whole matter is discussed by Blanchard (**13**), who also gave a list of species.

Finally, the above is only provisional, and personally I think it is, perhaps, preferable to state that the Spirochætes are annectant forms between strict Bacteria and strict Protozoa. This may savour of begging the question of the systematic position of the Spirochætes, but Nature does not set arbitrary boundaries marked off by hard and fast lines. In the evolution of any two distinct forms numerous transitional ones occur, and classification is only intended to mark the state of our knowledge for the time being.

Summary and Conclusions.

The new features and main conclusions of the memoir may be summarised seriatim thus:

1. In studying such minute and attenuate organisms as Spirochætes it is most necessary to observe them as far as possible in their natural media, and especially to study their movements.

2. A knowledge of their movements, together with that of their structure as revealed by stained preparations, should determine whether the organism in question is a Spirillum or a Spirochæte. The dispute as to the generic position of the pathogenic organism of relapsing fever, S. obermeieri, seems to me rather futile unless its movements and structure are carefully correlated.

It is not sufficient to rely on stained preparations alone.

3. For organisms like S. balbianii and S. anodontæ fixation is best done wet by means of osmic vapour.

4. The motion of these two Spirochætes in question is resolvable into at least two components: (i) a vibratory motion of flexion of the body mainly for progression, and (ii) a spiral or corkscrew movement of the body as a whole, due to the winding of the membrane. The corkscrew motion is especially well seen in S. anodontæ, which has pointed ends.

5. The membrane consists of a spirally wound lateral extension of the ectoplasmic periplast. It is a characteristic feature of the genus Spirochæta, as now defined. It is composed of approximately longitudinally arranged fibrillæ, the "myoneme fibrillæ." The border of the membrane is thickened, and stains well with chromatin stains. This thickened chromatic edge probably acts as a strong myoneme.

6. This structure is a membrane and not a sheath (see pp. 30, 31). Its fibrillar nature is best shown with gentian-violet or iron-hæmatoxylin. It is not so well revealed by Romanowsky stains.

7. The membrane does not markedly undulate. It is the

principal agent in progression. Its myonemes set up transverse movements in the surface of the body, manifested as waves passing down the body in a direction opposite to that in which the organism moves.

8. In life, during active movements the membrane is closely contracted round the body, and is not easily seen except as a halo round the organism. It is more difficult to discern in S. anodontæ.

9. The so-called ciliate (flagellate) stages of S. balbianii, mentioned by Vlès (**11**), are really due to the rupture of the membrane, and the apparent flagella are really myoneme fibrils. No flagellate stage occurs in life. This is probably of far-reaching significance, and may explain the discrepancy in the accounts given of S. gallinarum by Borrel and Prowazek.

10. The nucleus is diffuse, and consists of a helix of achromatic substance in which small bars or rodlets of chromatin are disposed during the trophic condition. The helix apparently is slightly looser in periods of activity such as division; the achromatic substance is then more evident, probably containing scattered chromidia, while chromatin masses are seen along the turns of the spiral.

11. Longitudinal division is common. Transverse division also occurs, but far less frequently. This is to be expected in long thread-like organisms wherein the longitudinal stress is probably less than the transverse, the envelope being longitudinally striated.

12. A small mass of chromatin occurs at either end of the periplast of the organism. It may be termed a basal granule, and is one of the first structures to show signs of fission in forms about to divide.

13. Regarding the life-cycle, only asexual methods of multiplication, principally by longitudinal fission, are known with certainty.

14. It is interesting to note that Spirochæta balbianii was found actively swimming about in sea water in which infected oysters had been kept for some time.

These Spirochætes are gut parasites with a primitive habitat. The mode of infection of the Lamellibranchs by them seems to be a casual one, namely, by the mouth.

15. The systematic position of the Spirochætes is difficult to determine. They have affinities both with the Bacteria and the Protozoa. They are undoubted Protists. Personally, I rather incline to the Protozoal nature of these organisms, and consider that a provisional new class of the Protozoa, namely, Spirochætacea, might be instituted for their reception when our knowledge of them is a little more extended.

At this juncture it will be well to deal with one or two points relating to the Spirochætes discussed in this memoir, but not, perhaps, directly concerned with the body of the paper. These have been left over purposely until the end, and will be considered in the form of Appendices.

Appendix I.

On Some Points in the Chemistry of the Crystalline Style of Anodonta cygnea.

The functions of the crystalline style of Lamellibranchs have been much disputed. To discuss this question of function is hardly within the purview of this Appendix, but I may state that Mitra (**28**) has, in my opinion, given by far the best account of this structure, though, perhaps, his meaning is not always clear. Mitra began a chemical investigation of the style in Anodonta cygnea.

The substance of the crystalline style is incapable of diffusing through a membrane, and is proteid in nature. It is, therefore, a colloid. In order to determine its proteid nature the Xanthoproteic reaction was tried, and a reddish-yellow coloration was obtained with concentrated nitric acid and ammonia. Confirmatory tests were Millon's reaction (corrosive sublimate and nitric acid) producing a yellow pre-

cipitate—while a violet coloration resulted from a mixture of copper sulphate and potash (Piotrowski's test).

The style is soluble in water, giving a definite alkaline solution, not neutral as stated by Mitra. The style is also soluble in dilute solutions (up to 5 per cent.) of sodium chloride and Epsom salts (magnesium sulphate). Its concentrated solution in water coagulates on heating, and from such concentrated solutions it may be precipitated by nitric acid, and by alcohol. It is, however, insoluble in concentrated saline solutions, such as those of sodium chloride and magnesium sulphate, and is also precipitated by tannin. It is then a globulin. A portion of liver of the Anodon ground up in saline solution also gave these reactions, as did a piece of the "flêche tricuspide" of Poli, though, in these cases, the test-results were less marked.

Mitra states that the style is entirely soluble in water, and this has been queried by Latter (27). I find that a style of average size (about two inches long) is dissolved in from three to four hours in water from the medium in which the Anodon is kept. Occasionally complete solution may require from five to six hours. Mitra gives no statement of the time required for solution. During this period of solution the Spirochætes swim about actively.

Several styles from different mussels were added together to a little starch solution, and after reacting for some time—preferably two to three hours—gave a sugar capable of reducing a cupric solution to the cuprous state. An amylolytic ferment is, then, present in the proteid substance of the style.

The crystalline style, is, according to Mitra, secreted by the liver, but it may also be partially secreted by the yellow, granular, epithelial cells lining the stomach and intestine. I think these cells of the wall of the stomach undoubtedly secrete the "flêche tricuspide" or cuticular lining of that organ.

The style in the oyster, like that in Anodon, lies in the cæcal division of the anterior part of the intestine, separated

by a fold or "typhlosole" from that part of the intestine serving for the passage of the gut-contents, as described by Perrin (6) and Mitra (28) respectively. No useful purpose would be served by my merely reiterating their correct descriptions. However, I found that sometimes in the case of Anodon, when the style was seemingly absent, it was really far back near the posterior end of the cæcal compartment—this was the case in mussels that had not been feeding recently. This may be explained by the anterior part of the style having been used, or by the style during periods of starvation being pushed back into this hinder region of the cæcal compartment. The whole alimentary canal is lined by cilia, the movements of which might aid in the process. The yellow, colloidal, cuticular lining of the anterior part of the cæcum in such starving mussels was not found to contain Spirochætes. I always found the crystalline style to be clear and hyaline, though under the microscope its outer, denser layer was striated longitudinally, while its inner zone was more fluid.

Crystalline styles were found in fresh water mussels opened at all times, whether after feeding or not, as maintained by Latter (27).

Unfortunately I had not enough material to conduct a detailed chemical examination for the metals and acid-radicals likely to occur in the style, but by means of flame-tests it was found that calcium was present, imparting a yellowish-red coloration to the flame, and also potassium, giving a lilac or delicate violet flame-coloration.

In the case of infected oysters the crystalline style often was not present, but the gut contents of the cæcal region invariably dried or "separated out" in tree-like patterns. This was also the case in styles from mussels placed on a slide and dissolved in a little water, for this solution also "separated out" on drying in similar patterns.

The Spirochætes described in this paper live, then, in a globulin-containing medium in which is an amylolytic ferment.

Appendix II.

Some Remarks on Wave-like Motion in Organisms.

The transverse vibrations set up by the myoneme fibrillæ in the membrane of Spirochætes—while moving through the somewhat viscid, colloidal substance of the crystalline style, or the gut-contents of that region containing the dissolved substance of the style—which vibrations were discussed in a preceding section, resemble those of light waves in the ether. The ether is, I believe, considered by physicists to be a viscid stuff and may be compared with the colloidal substance of the crystalline style. The transverse vibrations of the light waves in the ether bring about longitudinal forward motion, just as the transverse vibrations of the myonemes of the membrane set up pressures inwards on the organism, and so propel the organism longitudinally forwards.

Regarding the sudden reversal of movement in the Spirochætes this might be due to an accumulation of electrical charges at the anteriorly directed end or "pole" of the organism and its sudden liberation thereat. This spontaneous liberation of energy would induce or stimulate movement in the opposite direction.

Surface tension might also aid in the movements of these organisms, but it varies with the temperature.

Possibly some of these statements may appear a little crude to physicists, but I think they offer legitimate lines for further thought and elaboration.

Appendix III.

On the Possible Formation of Myonemes in Spirochætes.

Chromidia are capable of active migration, and such migration may take place towards the poles of the organism. During such a period as in division, a number of chromidia may thus move forwards and concentrate at the ends to form

"basal granules." Hence a gap would occur between the "basal granules" and the rest of the chromatin helix, and such a cytoplasmic gap actually is seen in the organisms in question (see Pl. 1, fig. 5; Pl. 2, figs. 16, 27; Pl. 3, fig. 22).

Possibly by a transverse outward migration of chromidia and subsequent concentration along lines in the periplast or its appendages, myonemes are produced. They might also be formed by chromidia from the "basal granules" migrating along approximately longitudinally disposed lines; and perhaps the thickened edge of the membrane is really finely continued into the basal granules, and might even be compared with the "marginal flagellum" of the undulating membrane of a Trypanosome.

In the case of Protozoa with a condensed nucleus and also possessing myonemes, like the Trypanosomes, the myonemes are formed from the "mantle fibres." The matter, however, is most difficult, indeed almost impossible, to decide in the case of such attenuate organisms as Spirochætes.

August 14*th*, 1907.

Addendum.

Since the foregoing was written several papers have appeared with a more or less direct bearing on the subject. Bourne (**32**) has given an excellent account of the crystalline style in Ænigma which confirms several of the remarks made in Appendix I. Bourne also recalls attention to Barrois' work, which Mitra had apparently overlooked.

Further notes by Schaudinn (**34**) on Spirochætes, especially S. plicatilis, have appeared, but no figures as yet. Lastly, I have just published a preliminary account (**33**) of my researches on Spirochætes in the form of an abstract of this memoir.

Swellengrebel's longer and illustrated memoir (**35**) appeared in July. Herein, the author notes that the greater number of oysters examined by him did not contain a crystalline style

(cf. p. 7 of this memoir). He describes very briefly the movements of S. balbianii, but does not account for them. His fixation was strong formalin, which I have not found to be so useful as osmic vapour or even methyl alcohol. No myoneme fibrillæ are figured in Swellengrebel's paper. Although allowing that the spiral filament of chromatin divides in a longitudinal plane, yet the organism is said to divide transversely. This is probably explained on reference to my fig. 19 (Plate 3) and p. 46, paragraph 3.

Moreover, it is interesting to look up the older writers on Bacteria, such as Zopf (**36**), for their views on Spirochætes. Zopf, although believing in pleomorphism and in the absence of a nucleus in Bacteria, yet notes that bipartition may take place in two or even three directions, though usually only in one (cf. this memoir, p. 45). Regarding locomotion he notes that cilia may be present in some forms (though apparently not in Spirochætes) as eversible projections through the envelope, and that, in addition, there is an oscillatory movement.

In conclusion, I hope I may be permitted to state that the observations recorded in this memoir, and the interpretations of them herein set forth, are the result of protracted and earnest endeavours to grapple with probably the most difficult group in the whole range of Protozoology, a group of the utmost morphological and economic importance, all previous accounts of which are most conflicting or only deal with minor points. I trust then, that this memoir of mine may be thus considered and lead to further investigations of organisms like S. obermeieri and S. gallinarum of relapsing fevers, along the lines and from the important points of view herein suggested.

University College, London;
October, 1907.

The following list of References to Literature is not claimed to be a complete one on Spirochætes—but only a list of many of the more important memoirs thereon. Further

references are given in some of the papers quoted, but I have, I think, given a complete list of references to the Spirochætes of Lamellibranchs.

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Note (see p. 45).

While revising the proofs of this memoir, my attention has been drawn to "A Note on the Morphology of Spirochæta duttoni," by the late J. E. Dutton and J. L. Todd, published in the 'Lancet' for Nov. 30th, 1907, pp. 1523-5. These authors find that S. duttoni "may multiply by direct division longitudinally or transversely," and that an undulating membrane is present (see my footnote on p. 34 of this memoir).—December 3rd, 1907.

EXPLANATION OF PLATES 1—3,

Illustrating Mr. H. B. Fantham's paper on "Spirochæta (Trypanosoma) balbianii (Certes) and Spirochæta anodontæ (Keysselitz): their Movements, Structure, and Affinities."

All figures were outlined with Zeiss's camera lucida, using the 2 mm. or 3 mm. apochromatic homogeneous immersion objectives, and compensating oculars 4, 8, 12, or 18 of Zeiss. Figures 1 to 23 are of Spirochæta balbianii; figures 27 to 40 of S. anodontæ.

PLATE 1.

Fig. 1.—Typical form of S. balbianii, with membrane close to the body, but one end curled. Absol. alc. fixation, Leishman stain. × 1500.

Fig. 2.—Parasite showing myonemes in the membrane. The thickened edge of the membrane is turned (folded) over at one point. Myoneme fibrils, some of which are perhaps slightly thickened, are seen on the periplast of the body at *my*. Iron Hæmatox. × 1500.

Fig. 3.—Parasite showing myonemes plainly. In the middle of the body the "zigzag" character of the nucleus is plainly seen. Long staining with Leishman's stain, and very careful focussing were necessary. × 3000.

Fig. 4.—Short form, with membrane closely attached to the body, especially visible along one side. Chromatin only indistinctly seen. Iron Hæmatox. × 1500.

Fig. 5.—Specimen showing myonemes in the membrane. The nucleus appears irregular, and is not clearly distinguishable throughout the length of the organism, possibly on account of the darkly staining background (Iron Hæmatox. used). A spiral nuclear filament is seen for a short space in the middle of the body. A well-defined basal granule (*b.g.*) occurs at each end. The spiral winding of the membrane is also well shown where it crosses both above and below the body. × 2000.

Fig. 5a.—Parasite with well-marked membrane, body-myonemes, and scalariform condition of the nucleus. The membrane exhibits numerous twists and folds. Myonemes—separate from those of the membrane—show plainly on the body at *my*. The myonemes of the membrane, indeed, are scarcely visible. A basal granule (*b.g.*) occurs at either end, and a centrosome (*cent.*) at one end. Gentian-violet. × 2500.

Fig. 6.—Specimen much coiled on itself (into a sort of S-shape), and there

is marked torsion of the body axis. The middle portion of the membrane is well seen, but no nuclear detail appears. Iron Hæmatox. × 1500.

Fig. 7.—Short form, but showing the membrane distinctly. No nuclear detail discernible. Such specimens in life, or after unsuccessful staining, may appear as if devoid of a membrane. But a membrane is really present in all such forms. Iron Hæmatox. × 1500.

Fig. 8.—Similar form of parasite, with membrane close to the body. In this case the scalariform character and arrangement of the chromatin masses are clearly seen after staining with gentian-violet. × 1500.

Fig. 9.—So-called "flagellate" ("ciliate") stage, from a preparation made at Roscoff, fixed quickly with absolute alcohol and stained with gentian-violet. The chromatin masses, arranged as rodlets, are clearly seen, while the "flagella" are myoneme fibrils from the frayed and ruptured membrane, as can be observed under exceptional conditions during the breaking of the membrane in life. × 2000.

Fig. 10.—Specimen stained with Leishman's stain, and apparently devoid of a membrane. However, with very careful focussing, under a magnification of 2000 diameters (as sketched), fine, faintly pink-staining myoneme fibrils are seen, resulting from the disintegration of the membrane. A so-called "flagellate" form.

Fig. 11.—A very interesting specimen, showing apparently an attempt at longitudinal division of the membrane and subsequent rupture of one of the portions of the divided membrane. A "basal granule" is present at either end, indistinctly divided into two. The helicoid character of the nuclear filament is well seen in parts. Myonemes of the unruptured portion of the membrane and so-called "flagella" in the ruptured parts occur together. Gentian-violet. × 2000. Myonemes are found in the periplast of the body at *my*.

PLATE 2 (Left Half).

Fig. 12.—Short specimen, with one end curled in a characteristic manner; the membrane is closely applied to the body, except at one point near the middle.

Fig. 13.—Early division stage. The membrane has already divided longitudinally. The nuclear helix exhibits a state of activity (as in division), showing its spiral character in parts, while near the "lower" end the rod-like chromatin masses have already become dumb-bell shaped. Basal granules not clearly stained. Interlacing appearance of the myonemes of the two membranes seen in the middle of the specimen. Gentian-violet. × 2000.

Fig. 14.—Specimen in early stages of longitudinal fission, as in the last figure, but the stage is very slightly later as judged by the nuclear figure. The chromatin masses have become dumb-bell shaped and divided at the left

hand end, while in the remainder of the body the chromatin is disposed in rodlets. The basal granules are not well stained. Gentian violet. × 1500.

Fig. 15.—Parasite in process of longitudinal fission, the membrane having already divided into two daughter membranes. Spiral nuclear filament well seen in the middle region of the body. Basal granules faintly stained, showing division. Gentian-violet. × 1500.

Fig. 16.—Longer, rather straight form showing division of membrane. Spiral nuclear filament at one end, where the basal granule has just divided. Basal granule has not yet divided at opposite end (which is unusual). Gentian-violet. × 1500.

Fig. 17.—Longitudinally divided parasites in process of separation. Two basal granules can be seen at the undivided end, while there is a single basal granule at the free end of each daughter form. Nuclear detail not clearly distinguishable. Iron Hæmatox. × 1000.

PLATE 3 (Left Half).

Fig. 18.—Daughter forms, still attached at one end, and vibrating in unison, arranged like the legs of a compass. A vacuole is seen in the cytoplasm of the common area of attachment of the two parasites before the longitudinal division is completed by the final separation of the daughter individuals. Leishman's stain. × 1000.

Fig. 19.—Long form, with the membrane discontinuous in the centre, at any rate along one side, where there occurred a vacuole-like area with clear and sharp contour. The periplast was continuous over the gap, at any rate definitely on one side. This seems to be a case of transverse division. The original basal granule at either end of the transversely dividing form has in each case divided into two. The whole nucleus seems in process of division longitudinally, or preparing for such division. Each "transversely produced" daughter form seems about to divide immediately by longitudinal fission. Further, reddish or pinkish dots are seen in the periplast on the edge of the body in at least two places, and these are apparently myonemes —slightly contracted—in the surface of the periplast of the body proper. Billet's stain. × 2000.

Fig. 20.—Specimen, stained with Billet's modification of the Romanowsky stain, showing the edge of the membrane folded over in the middle portion. Basal granule at each end. × 1800 approx.

Fig. 21.—Somewhat coiled specimen, which is small, but nevertheless shows membrane and basal granules. Billet's stain. × 1000.

Fig. 22.—Typical parasite, probably killed (with osmic vapour) while in rapid forward motion, judging from wave-like outline of body and membrane. Basal granule (*b.g.*) clearly distinguishable at either end, and perhaps a

"centrosome" (*cent.*) as marked. Billet's stain followed by tannin orange. × 1000.

Fig. 23.—Attenuate form, apparently young, as division stages, and many attenuate parasites occur in this preparation. Membrane probably closely attached to body, only clearly seen at one point. In this, and the parasite sketched in the preceding (fig. 22), the thinner chromatin rodlets are probably at a deeper focus. Leishman's stain. × 1500.

PLATE 2 (Right Half).

Figs. 24, 25, 26.—Spirillar forms from hind gut of cockroach, with terminal flagella and diffuse (faintly spiral) nuclei. Gentian-violet. × 1500.

Fig. 27.—Typical specimen of S. anodontæ, with spirally wound membrane, showing myoneme striations faintly, and in parts a spiral nuclear filament. There is a "basal granule" near the base of the fine process terminating the pointed ends of the body. Delafield's Hæmatoxylin. × 2000.

Fig. 28.—Parasite attached to débris at one end. Membrane only faintly stained, but chromatin rodlets clearly seen. Iron Hæmatox. and Orange G. (but the orange does not stain the parasite). × 1500.

Fig. 29.—Specimen showing well marked spirally wound membrane and basal granules, but chromatin rodlets a little indistinct. Gentian-violet. × 1500.

Fig. 30.—Preparation stained with Safranin and Licht-grün, but the latter stain did not colour the parasite. Membrane edge clearly stained, but chromatin thread indistinct. × 1000.

Fig. 31.—Specimen stained in a manner similar to the last, but the chromatin elements (rodlets) are brought out. Safr. and Licht-grün. × 1000.

Fig. 32.—Rather broad specimen of S. anodontæ showing basal granules. Gentian-violet. × 1500.

Fig. 33.—Parasite from crystalline style of Anodon, with coiled posteriorally directed end characteristic of a slowly moving form. Edge of membrane seen at side of the coil. Safranin. × 500.

Fig. 34.—Parasite with nucleus sharply stained, but membrane, which is close round the body, only faintly indicated. One end of body, on left, rather rounded—from which a fine process starts. Gentian-violet. × 2000.

PLATE 3 (Right Half).

Fig. 35.—Parasite with one end curled, showing basal granules, membrane with myonemes, and chromatin rodlets. Billet's stain and tannin orange. × 2000.

FIG. 36.—Specimen in which both ends are curled. Billet's stain. × 1500.

FIG. 37.—Parasite showing well-defined nucleus of chromatin rodlets, and the presence of a membrane. Billet. × 1500.

FIG. 38.—Parasite, killed and fixed, while rapidly moving, by osmic vapour. Exhibits numerous sinuosities in body outline and in winding of the membrane, which is a right-handed helix. Chromatin rodlets well stained. A rather long parasite. Billet's stain and tannin orange. × 1500.

FIG. 39.—Parasite moving less rapidly than the last when killed and fixed. Membrane rather closely adherent to the body. Billet's stain. × 1500.

FIG. 40.—Specimen in process of longitudinal division. The membrane has already divided into two, and the chromatin rodlets have divided each into two, the daughter chromatin masses being peripherally disposed in the parent form. That the basal granules are double can just be discerned. Billet and tannin orange. × 1500.

The pointed end of S. anodontæ, terminating as a fine periplastic process, staining pink (or chromatin-like) with Giemsa's or Billet's stains, is well shown in these forms (figs. 35—40). It hardly seems to be a strict flagellum, for it is stiff. It is, however, very small and fine.

The Structure and Life-History of Copromonas subtilis, nov. gen. et nov. spec.: a Contribution to our Knowledge of the Flagellata.

By

C. Clifford Dobell, B.A.,
Scholar of Trinity College, Cambridge.

With Plates 4 and 5, and 3 Text-figures.

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Introduction.

I believe there is no group of Protozoa which has yielded more interesting results from its investigation than that of the Flagellata. Even since the wonderful work of Dallinger and Drysdale was published, the flagellates have been invested with a fascinating uncertainty regarding their reproductive capacities. And, although this work has never been confirmed, the recent discoveries of Schaudinn and Prowazek have revealed the existence of life-histories which are in no way less remarkable than those described by the English investigators. Yet in spite of all this the flagellates —with the exception of the trypanosomes—are much neglected by protozoologists.

In so far as it indicated the sexuality of the group, the work of Dallinger and Drysdale has received confirmation. But the remarkable multiple fission and sporulation—including the formation of ultra-microscopic spores—which they saw have been seen by no one since. And hence, although we may indeed say that, as the result of recent work, "the pronounced scepticism of Klebs, Senn, and others with regard to the occurrence of a sexual process in this class . . . is now completely refuted," it is by no means legitimate to conclude that "the views of Kent and of Dallinger and Drysdale, are at length vindicated."[1] One form of "reproductive granule" described by Dallinger and Drysdale appears to be in reality a starch grain! And Stein's "endogenous reproduction," from the nucleus in euglenoids turns out to be due to the presence of a parasite. There is still room for much research on the monads. With the possible exception of "Monas dallingeri" (Sav. Kent), no uniflagellate monad has had its life-cycle worked out with any degree of completeness up to the present day.

Having found a monad of this kind which can always be easily obtained, and is very well suited for microscopic

[1] 'Zool. Rec. Protozoa,' 1904.

investigation, I took the opportunity of working out the details of its life-history as far as I was able to do so. The present paper is the outcome of this, and although my work is incomplete it contains some observations which are of sufficient interest, I believe, for publication. I hope to be able at a future date to decide several points which at present remain obscure.

In the later part of my paper I have given a very brief outline of some of the more important work which has been done on flagellate morphology and life-history. My reason for doing so is that it is impossible to discuss many of the problems connected with the flagellates without reference to recent work in this direction. And many important papers are in journals which are not readily accessible to the zoologist, and but few are in the English language. I am fully aware of the many omissions made from the literature of the subject, but my aim has been to contribute something towards our knowledge of the Flagellata, not to write a monograph on them.

Material and Methods.

Whilst I was working with the small parasitic Protozoa, which live in the gut of our common frog (Rana temporaria L.), and toad (Bufo vulgaris L.), I often found it necessary to keep the contents of the alimentary canal for several days, in order to follow the development of the contained animals. A few days after removal from the frog the fæces nearly always contained a small uniflagellate monad in great abundance. It is this monad whose life-history I am about to describe in this paper.

In order to obtain a suitable number of the monads for investigation I find it best to proceed as follows: A frog or toad is killed and its large intestine removed. The contents are then carefully expressed into a small, perfectly clean glass dish and covered over with a glass plate. As the fæcal matter thus obtained is usually too thick for microscopical

examination, it is diluted with a suitable fluid. Water will answer the purpose, or 0·75 per cent. NaCl solution. But in most cases I prefer to use a solution containing albumen, as the fixation of film preparations for staining is very much easier when albumen is present. I have found the solution used by Grassi and Schewiakoff (**23**) for investigating intestinal parasites to be very suitable. It consists of 20 cc. egg albumen, 1 gr. NaCl, and 200 cc. distilled water. Another 10 cc. of albumen improves the solution for films. If some of the diluted fæces be kept for about five days a large number of monads can easily be obtained.

Examination of the living monads was carried out in hanging-drop preparations, waxed round the edges, or in slides upon which a drop of the culture had been placed and covered by a coverslip—also carefully waxed round. I have also used the moist chamber of F. E. Schultze with success. The best results were usually obtained with hanging-drop preparations. These are also the most easy to make into permanent preparations—by removing the coverslip, spreading out the drop, and fixing.

In transferring drops of culture containing the monads to slides, etc., I always used a sterilised platinum loop or needle.

A word must be said about the saline albumen solution which I have just described. It does not keep for more than a week or two, and must always be filtered before use as it harbours numerous micro-organisms. One of the most constant of these is a small Amœba, resembling A. limax, Duj., which lives on the surface, where it forms a slight scum. These organisms crawl actively about for some time, and finally encyst.

Although with the illumination properly arranged, and with good lenses, etc., a very great deal of the structure and development of the monads can be seen in the unstained condition, nevertheless I have found that many of the changes undergone by the monads are more easily watched when they are stained intravitam. The intravitam stains which I have used are neutral red, Brillantkresylblau (Grübler), and

methylene blue. Neutral red has been the most generally useful. It does not stain the living nucleus, but will colour the cytoplasm a faint pink, so that the nucleus appears more distinctly by contrast. The food masses are coloured various shades of red, orange, and yellow (see p. 86). Brillant-kresylblau has also been very useful. It stains the cytoplasm a pale bluish or purplish colour, leaving the nucleus as a very distinct grey globule. Food masses take up the colour very strongly, many of them staining red or purple in a metachromatic manner. Methylene blue has been of but little service.

In making permanent preparations I spread out a small drop of the culture solution on a coverslip and then fix the moist film so made as quickly as possible. The most suitable fixatives are Schaudinn's sublimate-alcohol (2 : 1), used hot, and formalin (Schering—40 per cent. formaldehyde). The former has been especially useful. Osmic vapour is very useful for displaying the flagella, and good preparations can also be made after fixation in Hermann's solution.

By far the most useful stain is Heidenhain's iron hæmatoxylin. I use this alone, or sometimes counterstain with eosin or orange G. Other stains which have occasionally been of use are Delafield's hæmatoxylin, used very dilute, and Giemsa's stain. The latter is difficult to use, and untrustworthy, but it has proved of some service on special occasions —e. g. in cyst formation. Methyl green (used in the manner described on p. 83) has also been useful.

The permanent preparations were always mounted in balsam, except those stained by Giemsa's method, which were mounted in cedar-wood oil.

For investigating the minute anatomy of the animals I have successfully used the method devised by Schewiakoff (**45**) for ciliates. It consists in killing the organisms with osmic vapour and examining them in 10 per cent. soda solution. Many structures are rendered very clear by this method.

In examining the living animals I always used the 2·5 mm. apochromatic water-immersion of Zeiss (apert. 1·25), cor-

recting for the thickness of the coverslip by means of the correction-collar. For permanent preparations I used Zeiss's 3 mm. apochromatic oil-immersion (apert. 1·40), or less frequently the 2 mm. (apert. 1·40). Compensating oculars 2, 6, 12, and 18 were employed. I used a large Zeiss stand and artificial (incandescent) light.

I have always attached the greatest importance to the observations made on the living animal, stained preparations being used to check and amplify these observations.

Culture.—In a state of nature the frog usually deposits its fæces in the water or on damp earth. They must, therefore, be frequently diluted with water, so that a watery solution of the fæces is probably a normal medium for the monad. I attempted, however, to discover whether the monads could live in other culture-media. Unfortunately, I have not made an extended series of experiments in this direction, nor have I been able to discover any other natural habitat of the monad than that already recorded. I have succeeded, nevertheless, in keeping the monads for several days in a state of activity and frequent division in organic infusions of several kinds, and infusions of fæces of several different mammals and of a snake. An organism described as a "zoospore" was observed in infusions of cow-dung by Cunningham (11) in India. It bears some resemblance to my monad when seen under a low power.

Systematic.

It is not possible at present, owing to our ignorance of the life-histories of most flagellates, to assign this form to any very definite systematic position. Beyond doubt it belongs to the class Mastigophora, Diesing, and to the sub-class Flagellata (Cohn) Bütschli. From the morphology of the adult form it may further be referred to the order Euglenoidina, Klebs. So little is known of the various members of this order that it is difficult to decide upon the right family, sub-family, etc., which should include the form

under consideration. It is, indeed, premature to attempt detailed classification, for there can be no doubt that, with increased knowledge of flagellate life-histories, our present system will have largely to be recast. I would point out, however, that the creature should most probably be referred to the family Peranemida, Klebs, and sub-family Petalomonadina, Bütschli. Its nearest allies appear to be Petalomonas, Stein, and Scytomonas, Stein. Judging from the figures of this latter (S. pusilla, Stein) given by Klebs (**28**), Pl. XIV, fig. 9 a–d, there is considerable similarity between the two organisms. But very little is known of the various species of Petalomonas and Scytomonas, so that it is impossible to make any definite statements on the subject. I must be content, therefore, to leave the monad in its present unsettled position, awaiting the work of future investigators.

I propose to name this monad Copromonas subtilis, nov. gen. et nov. spec. The systematic position may therefore briefly be expressed thus:

Phylum.—Protozoa.
Class.—Mastigophora (Diesing).
Sub-class.—Flagellata (Cohn emend. Bütschli).
Order.—Euglenoidina (Klebs).
(? Family.—Peranemida [Klebs]).
(? Sub-family.—Petalomonadina [Bütschli]).
Genus.—Copromonas (nov. gen.).
Species.—subtilis (nov. spec.)

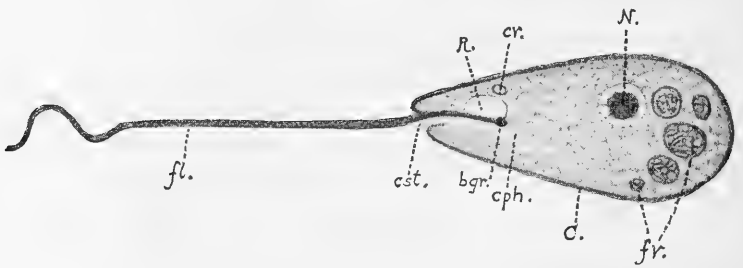
Structure.

The general anatomy of Copromonas is seen in the accompanying diagram (text-fig. A).

Although the organism, when seen under a low power, appears to be of a very simple structure—consisting merely of a globule of protoplasm with an anteriorly-directed flagellum—it possesses, in reality, considerable morphological differentiation. The body is more or less ovoid or pyriform,

and varies considerably in size. About 16 μ is an average length, but individuals are found of all sizes from about 7·5 μ to 20 μ. The breadth averages about 7–8 μ. The anterior ends bears a flagellum (*fl.*), in length usually rather greater than the body. This flagellum arises from a depression at the anterior end; the depression is a cytostome (*cst.*), or cell-mouth, and leads into a longitudinal tube, the cytopharynx (*cph.*). This extends backwards for a variable length, and is somewhat spirally disposed. It can be seen, in carefully stained preparations, that the flagellum runs along the wall of the cytopharynx for a short distance, and takes its origin from a basal granule (*bgr.*), which is strongly coloured by

TEXT-FIG. A.



iron-hæmatoxylin. The base of the flagellum is in intimate relation with a large vacuole-like space (*R.*), the reservoir. Into this latter a minute contractile vacuole (*cv.*) rhythmically discharges its contents. Sometimes two small contractile vacuoles are present, instead of the usual one.

The reservoir itself does not pulsate, but from the fact that it is sometimes absent it may be inferred that it periodically collapses, driving out its contents. No communication with the cytostome can be made out, however.

When the monad is at rest, either during division or when compressed by a coverslip, it can be seen that the contractile vacuole pulsates at the rate of once in about thirty seconds.

It will be seen from the diagram that the reservoir, on its pharyngeal aspect, is related to the root of the flagellar

apparatus. On account of the small size of the animal it is exceedingly difficult to be quite certain of the exact interrelations of these parts. At times the basal granule seems to be situated on the posterior part of the reservoir, from which the flagellum then arises. This arrangement somewhat resembles that seen in Euglena, as described by Wager (**52**), but in Copromonas no forking of the flagellar insertion can be seen. When such a condition exists the flagellum appears to cross the reservoir, thus lying for a short distance within this structure.

The nucleus (*N.*) lies somewhat posteriorly, and is not connected in any way with the flagellum, as is so often the case in flagellates (cf. Plenge [**32**], etc.). In the living monad it appears as a greyish, spherical vesicle, slightly more refractive than the surrounding cytoplasm. In stained preparations, however, it can be seen to possess the structure shown in the diagram. That is to say, it consists of a central, deeply-staining chromatic mass, surrounded by a clear zone which contains practically no chromatin. Surrounding this is an achromatic nuclear membrane. Achromatic strands unite the membrane with the central portion. I have found that the structure of the nucleus is most easily demonstrated as follows: A small drop of solution containing the monads is placed on a glass slide. A small drop of a solution of methyl green in 1 per cent. acetic acid is then placed in the centre of a coverslip, which is carefully lowered on to the first drop. The coverslip is then pressed firmly down, and the preparation examined with the water immersion. In such a preparation the central chromatic part of the nucleus is coloured green: the nuclear membrane swells out slightly and becomes very distinct. Many other details of the anatomy can be made out in this way.

It will be seen that the nucleus is of the second type distinguished by Prowazek (**36**) in Flagellata (see infra, p. 104).

The posterior region of the body is usually more or less filled with food masses. These are of varying sizes, and formed in the manner described below (p. 86). Outside the

entire body there is a well-developed cuticular layer (*c.*). Its presence is very well demonstrated in degenerating or macerated monads (see p. 103). It does not appear to be composed of cellulose, as it is stained a pale greenish-yellow with Schultze's solution. No striation is visible, in correlation with the non-contractility of this investment (see "Movements," infra).

As I have already remarked, there is no connection between the nucleus and the flagellum. But I may here call attention to the fact that in stained preparations a very distinct dark line is sometimes seen uniting the base of the flagellum to the nucleus. After examining a considerable number of monads which show this I am satisfied that it is really due to the cytopharynx, the animals having rolled over so that the cytopharynx appears to be in line with the flagellum, and to connect it with the nucleus, over which the cytopharynx has come to lie.

I have never seen any appearances which would lead me to suppose that the flagellum is "ciliated" in the manner described by Fischer (**20**) in Monas and Euglena—the so-called "Flimmergeissel" arrangement.[1] The flagellum, on the contrary, appears to be a perfectly regular and undifferentiated filament.

Movements.

Copromonas displays a very characteristic series of movements. Under ordinary conditions it draws itself slowly and evenly along by means of its flagellum. When undisturbed the monad uses only the anterior end of this organella for locomotion; the remainder, comprising about the posterior three quarters, remains rigid. Stimulation, either by shaking or other means, causes a vibration of the whole flagellum, as in the case of Peranema (see Verworn [**51**]). In turning,

[1] I think, with Plenge (**32**), that these appearances are due to foreign bodies adhering to the flagellum.

the flagellum is directed backwards along the body, and by forcible movement of the anterior end the body is tugged round. The only other movements which occur are rolling, to a limited extent, caused apparently by a screw-like movement of the flagellum, and a curious lurching movement which is every now and then observable. It usually occurs when the animal is about to turn, but it is difficult to see how it is brought about. After progressing steadily and gracefully forward for some distance in a straight line, the monad suddenly gives a clumsy lurch sideways; then, appearing to regain its equilibrium, it continues its course, usually in a different direction.

Intrinsic movements do not take place. Owing to the rigidity of the surrounding pellicle no "euglenoid" movements are possible. The pellicle is not contractile, so that the contours of the body remain constant. In contrast with many other flagellates there is never any tendency to become amœboid. Irregularity of shape is only seen in monads undergoing degenerative changes (see infra "Degeneration," p. 102).

Nutrition.

The method of nutrition is holozoic. There can be no doubt that the depression at the anterior end of this organism —the cytostome—is a true cell mouth. Into this structure food particles are being constantly introduced by the forward movement of the monad. Bacilli, micrococci, and minute organic particles of all sorts, which are very plentiful in the medium in which the monad lives, enter and pass along the cytopharynx, and are taken up by the protoplasm at the posterior end. Many of the larger bacilli, etc., which enter the mouth, and even pass along the pharynx, are not ingested but return to the exterior again. If a monad be carefully watched for any length of time it will be seen that most of the larger particles behave in this way, only the smaller being actually ingested. Occasionally an unusually large bacterium

makes its way into the cytostome, and gets its end stuck fast in it. In this case the monad may swim about for a considerable time with the rod sticking out of its mouth, presenting a curious appearance to the observer.

Subsequent changes in the ingested food masses are most easily seen in organisms stained intravitam with neutral red. It can then be seen that the following series of events takes place: At first minute particles are seen in the protoplasm at the bottom of the cytopharynx. They appear to lie freely in the cytoplasm, without any vacuole surrounding them. After a time the particles are found to have agglutinated, and are enclosed in vacuoles. Digestion now takes place, and the agglutinated masses are gradually eroded. By means of neutral red the different stages of digestion are very beautifully demonstrated. Ingested particles at first stain a bright red. In the food vacuoles the food bodies also take up the stain strongly, but they are coloured reddish-orange. Later stages are usually of an orange or yellowish hue; so that the posterior end of the organism may contain food balls of different colours of red, orange and yellow—the colour corresponding with the stage which the digestive process has reached. It may be inferred that the digestive juice is neutral in reaction, but becomes somewhat alkaline as digestion proceeds. The change of colour may also be due partly to reduction. (Neutral red becomes yellow with alkali, to which it is exceedingly sensitive, and is reduced to a colourless leuco-product.)

The whole process, as I have observed it in Copromonas, bears a close resemblance to that described and figured in Paramœcium by Prowazek (**33**).

Stokes (**49**) has described the ingestion of food by means of a "mouth" in Petalomonas, a genus which, as I have already pointed out, is probably closely allied to the form under consideration. The method of feeding seems to be identical in the two genera.

As regards the larger euglenoids, much doubt still exists regarding the function of the so-called "mouth." Although

the experiments of Saville Kent (**24**) appeared to have definitely proved that food was ingested at this aperture in Euglena, the recent work of Wager (**52**) throws some doubt upon the matter again. From Wager's observations it is clear that at least one function of this structure is connected with excretion—that is to say, it serves as a duct for the reservoir. Khawkine (**26**) has made the suggestion that liquid food enters by the mouth, but the evidence in support of this is by no means conclusive.

In many monads (e. g. Oikomonas, etc.) food is ingested at any point on the surface of the body. This, however, never takes place in euglenoids such as Copromonas, which possess an external cuticular covering.

Life-cycle.

The life-cycle may conveniently be considered in two periods—a period of asexual multiplication, and a period of conjugation and encystment. These two periods are not sharply separated from one another, but overlap—that is to say, in any given culture some of the monads will finish conjugation before others begin.

The first period of the life-cycle is of variable length, and is made up of a very variable number of cell generations. It is, therefore, impossible to make any definite statement of the duration of this period. In cultures made in the manner described on p. 77 the following is the course of events frequently pursued, and may be taken as a fair average. But it must be remembered that it is only an approximation to the truth—not an invariable sequence of phenomena.

1st day. Culture made; no monads; a few cysts found after careful examination.
2nd ,, No monads.
3rd ,, No monads.
4th ,, A few very small monads.
5th ,, A good many monads, many dividing.
6th ,, Monads in large numbers, actively dividing.

7th day. Monads plentiful, many dividing and a considerable number conjugating.
8th ,, Many monads conjugating, some encysting and some dividing.
9th ,, } Conjugation practically finished; a few dividing;
10th ,, } encystment.
11th ,, }
12th ,, }
?
?
&c.

Sometimes the life-cycle extends over about seven days; at other times it may take more than twice as long.

(1) Asexual multiplication.

This takes place by means of longitudinal division, and may easily be observed in the living animal. As a rule division is proceeding most actively in cultures about a week old. By fixing and staining film preparations at this time a large number of specimens showing all stages of division may be obtained with considerable ease.

General account of division (see Pl. 4, figs. 1–10).—The monads grow to a large size before division. The first sign of the onset of the process is observable in the locomotor apparatus. It is seen that the animal (Pl. 4, fig. 1), which was actively swimming about, is becoming sluggish in its movements. After a short time it comes to rest completely, and the flagellum displays slow coiling movements (fig. 2). Gradually the movements become slower and slower, and at the same time the flagellum gets shorter and shorter, and is finally completely drawn in (fig. 3). During this process the nucleus has elongated, and now appears as a bright band stretching across the cell. After watching the motionless monad for a few minutes the observer will see two minute peg-like outgrowths appearing at the anterior end. These are the new flagella, which grow up side by side, and very

soon begin to writhe about (figs. 4 and 5). As they increase in length they exhibit the characteristic movements of these structures, and the dividing organism again becomes motile. A cleft has meanwhile appeared between the bases of the flagella. It extends backwards slowly, cutting the reservoir in two as it does so. The nucleus becomes completely parted into its two daughter-products, and by the cleft gradually extending to the posterior of the body two daughter-individuals are formed, and subsequently break away from one another (see figs. 9 and 10). The whole process lasts twenty minutes or thereabouts.

The foregoing is a general account of the way in which longitudinal division is effected. I will now describe in detail the manner in which the various organellæ of the monad are doubled in the formation of daughter-individuals.

Details of division.—These must be considered in the case of (A) the nucleus, (B) the flagellum, (C) the cytostome, (D) the reservoir or contractile vacuole.

(A) The nucleus.—(Pl. 4, figs. 6–10; Pl. 5, figs. 34–40). Nuclear division is effected by a kind of amitosis. It thus differs from the phenomenon which has been observed in all other members of the order Euglenoidina which have been accurately investigated. (See infra, p. 104).

In the living organism no details of division can be seen in the nucleus. It can be seen to elongate, and become constricted into two—as shown in figs. 1–5, Pl. 4—but beyond this nothing can be made out. However, in preparations stained with iron-hæmatoxylin, and carefully differentiated, the following details of division may be observed (see Pl. 5, figs. 34–40). Before division the nucleus consists—as already noticed—of a central chromatin-containing body, ("Innenkörper") surrounded by a clear, non-stainable zone ("Kernsaftzone") bounded by the nuclear membrane. Achromatic strands cross the clear zone, connecting the nuclear membrane with the central body (fig. 34). In strongly differentiated iron-hæmatoxylin preparations the central body appears to consist of an achromatic substance in which

chromatin is suspended in the form of granules of variable size.

The first phase of division is characterised by the nucleus becoming elongated and somewhat fusiform (fig. 35). The central body appears to throw off granules of chromatin at opposite poles. A little later the fusiform shape gives place to a more or less oblong form, like a short, blunt rod (fig. 36). Enlargement of the ends of the rod next takes place, with the formation of the characteristic dumb-bell figure (fig. 37). The chromatin masses itself at the ends of the dumb-bell, the intermediate portion becoming band-like and staining a lighter tint (fig. 38). Minute chromatin granules may be seen scattered throughout. The two ends of the dumb-bell then undergo increased differentiation and separation, becoming rounded off, and containing the aggregated chromatin elements of the nucleus. For some time these daughter-nuclei remain connected by a fine filament as in fig. 39. Finally the filament disappears, and two completely formed daughter nuclei are left (fig. 40).

A general discussion of the nuclear phenomena will be found on p. 103. I will here merely call attention to the fact that division is amitotic; there are no differentiated chromosomes, no extra-nuclear division centres. In this respect it resembles that of some rhizopods (cf. for example, the Leydenia-phase of Chlamydophrys).

(B) The flagellum.—(Pl. 4, figs. 2–5; Pl. 5, figs. 41–46). A description of the manner in which the new flagella are formed, as seen in the living monad, has already been given (p. 88). The finer details of the process can be made out only in stained preparations. The flagellar insertion is most clearly seen in osmic vapour preparations (fig. 41). In these the basal granule is very distinctly seen, lying in the wall of the cytopharynx against the reservoir. As we have already seen, the flagellum is retracted before division. In stained specimens it can further be seen that it gradually disappears in the direction of the basal granule, until only this structure is left (figs. 42 and 43). The basal granule

then divides (fig. 44), becoming dumb-bell shaped, and finally being constricted into two daughter-granules. From each of these a new daughter-flagellum springs up (fig. 45), and on reaching the surface becomes visible as the little peg-like structure—the shoot which develops into the flagellar stem —which I have already described in fig. 4. A corresponding stained stage is seen in fig. 46.

The manner in which flagella multiply has not been made out in many organisms. Dallinger and Drysdale (**12**), James-Clark, and others were of opinion that new flagella arose from the splitting of the old. On the other hand, Pelletan (in Dinobryon) and Klebs (in Euglena [**27**]) state that the new flagella arise by a new growth—one daughter-cell taking the old, the other the new. The process seen in Copromonas is of interest in connection with the morphology of the basal granule (see infra, p. 106). It is probable, however, that the flagella divide differently in different species.

(c) The cytostome.—The doubling of this structure is exceedingly difficult to observe. Even in the active adult it is often hard to distinguish, although it becomes more evident in monads which have been flattened out. From a number of observations on living animals and permanent preparations I believe that the cytostomes of the daughter-organisms are both new growths, the old cytostome apparatus having degenerated.

Before division the cytostome and cytopharynx are visible as a depression from which a dark line extends backwards (Pl. 4, fig. 1). As division proceeds this line becomes less distinct (figs. 2 and 3), and finally disappears. When the new flagellar rudiments first appear they are seen to be growing out of little pit-like depressions (see figs. 8 and 9, Pl. 4, and fig. 46, Pl. 5), which gradually penetrate the cytoplasm of the daughter-cells. These inpittings are the new cell-mouths and their continuations the new cell-gullets.

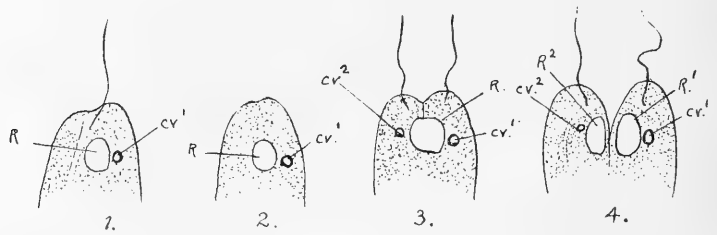
As a rule, it appears to be the case among flagellates that one of the daughter-monads retains the old cytopharynx, etc., while the other develops a new one. This happens, for

instance, in Trichomastix lacertæ, according to Prowazek (39). But really very little information is to be gleaned from the literature on the subject.

(D) The reservoir and contractile vacuole.—Owing to the circumstance that the monad is quite motionless during the doubling of these structures I have been able to observe the process with considerable accuracy. It can be most easily described with the aid of a diagram (see text-fig. B).

Before division the monad is seen to possess one reservoir (*R.*) with a single adjacent pulsating vacuole (cv^1). In fig. 2 the flagellum has been drawn in, and the cytopharynx has disappeared. Later (fig. 3) the two new flagella have made

TEXT-FIG. B.



their appearance and a cleft can be seen between them. On the left of the reservoir a new contractile vacuole (cv^2) has suddenly made its appearance, the old one (cv^1) remaining in its original position on the right.

The interflagellar cleft gradually extends backwards (figs. 3 and 4), and as it does so it completely halves the reservoir—one half going to each daughter-monad. At this stage the new cell-mouths are distinctly visible, lying over the vacuole apparatus (fig. 4, and see also Pl. 5, fig. 46).

During the whole of this process the vacuole (or vacuoles) continue to pulsate at a rate of about twice a minute.

I have described this process in some detail, because so little appears to be known about it in most flagellates. Division of the contractile vacuole has been described by some writers, but I think this is very doubtful. For the

majority of Flagellata, we can still say with Senn (**47**): "Über die Art der Vacuolenvermehrung wissen wir nichts näheres."

Transverse or multiple division has never been observed. A large number of cases of the former which have been described among flagellates are almost undoubtedly to be regarded as late stages in longitudinal division. There can be no doubt that it does occur in some forms, however, e. g. in Oxyrrhis. And amongst the Chlamydomonadina, according to Dangeard (**14**), division may be either longitudinal or transverse, the result being dependent upon the position of the achromatic spindle during mitosis. The plane of division is at right angles to this; and the position of the spindle itself is determined by the relative positions of the cell-protoplasm and the chloroleucite.

After longitudinal division has continued for a period varying from about two to six days, a considerable number of the monads will be found to be conjugating. I will therefore now describe this process and its sequelæ.

(2) Conjugation and Encystment.

The conjugating individuals are indistinguishable from their forerunners. Every monad, apparently, is a potential gamete. No difference, therefore, exists between the gametes themselves—that is to say, they are isogamic, displaying no sexual differentiation. It is true that occasionally one of the conjugants appears to be distinctly smaller than the other. But then it must be remembered that in cultures of the monads a great variation in size is often observable, and it is most probable that size-variation in this case is merely an expression of individual differences in rate of growth and food assimilation.

Conjugation may be easily watched in a hanging-drop preparation, though it is difficult to make out anything of the nuclear phenomena by this means, owing to the activity of the monads. The process, as seen in the living animals, may

be most easily described, I think, by recording a typical case (see Pl. 4, figs. 11–16). The first thing to be seen is the approach of two monads to one another. Hitherto they have been swimming about apparently at random, but they now draw near and come in contact by their anterior ends (fig. 11). Each monad appears to be normal, possessing one nucleus, reservoir, etc. For a few moments they swim about merely touching one another, and gliding over one another to some extent. But it is soon seen that an actual adhesion is taking place, so that the monads become firmly united at their anterior ends. Both the flagella continue to move actively, and very often get entangled.

After swimming about for some minutes in this manner it can generally be made out that one of the flagella is becoming shorter. At the end of a quarter of an hour this is usually very evident, and a little later, or perhaps even now, one flagellum is completely drawn in. Fusion is extending backwards, so that the conjugants have the appearance of one large, bilobed monad, rather than of two applied to one another (fig. 12). Active movements still occur, and further fusion is seen slowly to be taking place. In about half an hour more the monads present an appearance like that seen in fig. 13. It appears as though one monad absorbs the other; for a little later they have the appearance, shown in fig. 14, of one monad bearing a projecting process on its side. Still later the fused individuals present the appearance of one somewhat asymmetrical organism (fig. 15), only differing from the ordinary monads in being slightly bilobed at the posterior end. Sooner or later the remodelling is completed, and an organism exactly like an ordinary monad is formed, though it is usually noticeably larger, and sometimes can be seen, after careful examination, to contain two nuclei in place of the usual one.

The subsequent history of these binucleate monads is not always the same. Development may proceed along one of two lines: (1) The animal may encyst, or (2) it may continue to feed and divide longitudinally, just like an ordinary indi-

vidual. In the first case (1), the animal gradually becomes more rounded in shape, and decreases considerably in size. The flagellum is finally drawn in, and a very delicate cystic membrane is formed. A considerable time elapses during these changes, and I have never been fortunate enough to be able to observe the changes which occur in the living animal inside the cyst. These can be very clearly made out in stained specimens, however, and are described below.

The reason why the contents of the cyst are so difficult to distinguish is probably to be sought in the manner in which the reduction in size is brought about. I think this is probably effected by the protoplasm giving up part of its water. By doing so the cytoplasm would, for a time at any rate, become relatively denser, and so render the nucleus less distinct.

It is not easy to see the cyst when first formed. It is only visible as a pale border to the rounded-off cell, and is not easily distinguished from a mere optical effect which makes a halo appear round a small, brightly illuminated object. The cysts appear more distinctly after some hours' time, and seem to be composed of a soft, gelatinous substance. I have found that the most rapid way of demonstrating their presence at this and earlier stages is to proceed as follows: the hanging-drop containing the cysts is carefully spread out, after removing the coverslip, and allowed to dry rapidly. It is then fixed for ten minutes in absolute alcohol, and stained for ten minutes in Giemsa, then rinsed in water, blotted with a cigarette paper, and mounted in cedar oil. By this method the cysts are stained pink, the protoplasm inside dark blue, with the nuclei sometimes visible as dark blue, purple, or red bodies.

The gelatinous cysts, in the normal course of events, become hard and slightly yellowish in colour. They are not uncommonly encrusted more or less completely with the minute organic particles which abound in the fæces cultures. There is a good deal of variation in the shape of the cysts, some of the commonest forms encountered being shown in figs. 17, 18,

and 19, Pl. 4. They are roughly spherical, ovoid, or sometimes of the form shown in fig. 19, with a notch at one end. This notch marks the former position of the cytostome. In fully-formed cysts the nucleus can be clearly seen as a refringent sphere, lying in the middle of the protoplasm.

The second case (2), in which the zygote does not encyst, is of considerable interest. After swimming about for a time, the monad—which, although really a zygote, is indistinguishable from the asexually-reproducing animals—comes to rest and divides in the normal way. It can be seen to contain but one nucleus (which stained preparations show to be derived from the fusion of the two gamete nuclei), although the manner in which this single nucleus arose I have not been able to observe satisfactorily in the living animal. On one occasion when I carefully watched the conjugation of a pair of monads and their subsequent development I found that three hours and ten minutes elapsed from the time when complete fusion of the gametes had taken place until the zygote began to divide longitudinally.

The zygotes and their descendants appear to be able to continue dividing for a considerable period, though I cannot say definitely how long. In cultures in which most of the monads appeared to have conjugated—though one cannot be certain that all have done so—I have often found a few dividing individuals a week or ten days afterwards. In the end, however, the majority of these appear to encyst, the flagellum being drawn in and the cyst wall formed as in the case of the monads which encyst immediately after conjugation. (See Pl. 4, figs. 31–33).

As far as I am aware there is but one flagellate monad which has had ascribed to it the ability of continuing to lead an active life after conjugation. I refer to Dunaliella, one of the Phytomonadina, whose life-history has been investigated by Teodoresco (**50**). He describes the fusion of isogametes. One loses its flagella and is absorbed. Under favourable conditions encystment does not follow fusion, but the monads remain active. The nuclear phenomena have not been

elucidated, and I am unable to find any proof that the zygotes continue to divide like ordinary monads. Teodoresco does not appear to have followed out a pair of conjugants until the zygote which they produced divided, but he is probably correct in supposing that this takes place. "Si cela est vrai," he remarks, "ce serait alors le seul cas connu jusqu' à présent d'une zygote, provenant de l'union de deux gamètes mobiles, qui dans les conditions favourables de vie, ne passerait pas à l'état de repos." That such a condition actually does exist in Copromonas there can be no doubt. For, as I have already said, I have watched the living animals from the beginning of conjugation to the completion of longitudinal division in the resulting zygote.

I have succeeded in following out the nuclear phenomena which occur at conjugation in permanent preparations only. I will now give the results which I have obtained from a study of these preparations, which were made in the manner already described (see Pl. 4, fig. 21–33).

Before conjugation the nucleus appears to be quite normal, like the resting nucleus already described. The nuclei of both conjugants remain in this state until a considerable degree of cytoplasmic fusion has taken place and one flagellum has completely disappeared (figs. 21, 22). Each nucleus now divides, going through all the stages which I have already described in the process of asexual multiplication. The nuclei do not always divide simultaneously (cf. fig. 23). Even before division is complete it can usually be seen that one of the daughter-nuclei stains more palely than the other. This pale nucleus is a reduction nucleus, and it very soon degenerates and breaks up. In fig. 24 a pair of conjugants are seen in which both the nuclei have divided, so that there are now four nuclei present. Of these four, two (the lower two in the figure) are reduction nuclei, and may be recognised by their lighter colour. I should point out that the nuclei here appear rather larger than they really are, as the specimen has been slightly flattened out on the coverslip.

So far as I have been able to make out, only one equal

division of the nucleus takes place in the manner just described. But in monads in a later stage of fusion small granules of chromatin can often be seen apparently emerging from the nucleus. At first these appear as little projections from the central chromatin mass; but later, as they become separated, they are seen as small granules attached by a slender stalk to the central mass (see fig. 25). Finally, they become completely separated, and degenerate in the cytoplasm. This process might also be described as a heteropolar division. It appears to occur in the organisms after the first reduction division has taken place, for the degenerate fragments of the first reduction nucleus are usually to be seen in the cytoplasm (see fig. 25).

It is impossible to say whether a single reduction occurs in this manner, or whether more than one granule is extruded from the nucleus, on account of the fact that small broken-up bits of the first reduction nucleus are usually lying about in the cell. I can only say that at least one small nuclear mass is eliminated in this way. Personally I believe that only one granule is formed in each case, but I cannot prove that this is so, as I have never succeeded in watching the process in the living monad.

By the time that the nuclear reductions are completed the two conjugants have become very largely fused with one another as regards the cytoplasm (fig. 26). The two reduced nuclei now approach one another, and the nuclear membranes come in contact. If the fate of the zygote is immediate encystment the flagellum is drawn in (fig. 28) and a cyst wall formed in the manner already described. Inside the cyst, therefore, one sees two nuclei in contact with one another, as shown in fig. 29. A process of fusion between the nuclei then takes place in the cyst (fig. 30), so that ultimately the cyst contains but a single nucleus (fig. 33). The nuclear membranes first break down at the point of contact, and the central chromatin masses finally fuse. When spherical the cysts have an average diameter of 7 μ–8 μ.

The nuclei come together and fuse in the same way in those

zygotes which continue to lead an active life instead of encysting. But in this case, of course, the fusion of the nuclei is accompanied by an external remodelling of the cytoplasm (see fig. 27).

It is a matter of some difficulty to make out the fate of cell-organs other than the nucleus during conjugation. From its behaviour during division the basal granule of the flagellum might be thought to play some part in the process of conjugation. But although I have devoted a good deal of time and care in endeavouring to discover whether the basal granules fuse during conjugation I am still quite ignorant on the subject. Appearances suggesting a fusion are sometimes seen, but it is impossible to attach much importance to them on account of their very small size and the numerous other granules which often fill the cytoplasm. In many stages, indeed, I have not been able even to distinguish the basal granule of the flagellum which has been retained.

Regarding the reservoir and cytostome I am still uncertain. It appears to me that in both these structures one is absorbed during conjugation, whilst the other is retained and forms the permanent organella in the active zygote. Fusion does not appear to take place between the reservoirs, but one apparently collapses, leaving the other one functional. Two reservoirs may sometimes be seen until quite a late stage in conjugation (cf. Pl. 4, fig. 26).

The food masses of both the conjugants get mixed up in the zygote. They are all absorbed before encystment, so that the cysts have clear protoplasmic contents. I have not been able to follow out the fate of the basal granule in the cyst.

I have observed an abnormal fusion of three monads, but have never succeeded in tracing out the result of such a union. This condition is depicted in Pl. 5, fig. 47. It can be seen that two of the flagella have disappeared, only the basal granules remaining. There is no reason to suppose that complete conjugation ever occurs between more than two monads. But the observation is of some interest from the fact that a similar "dreifache monströse Kopulation" has been seen in

Polytoma by Prowazek (**36**). Dallinger and Drysdale (**12**) also stated that more than two monads might unite, previous to sporulation, in Bodo, although this has never been confirmed.

The points of special interest in the conjugation and consequent events in Copromonas are the nuclear reduction and the fate of the zygote. Some general remarks on the former subject will be found on p. 111. I will now pass on to the later history of the cysts.

Development from the Cysts.

Cysts formed in either of the ways described above appear to have exactly the same destiny. They are able to withstand drying for a considerable length of time, and do not liberate their contents until they reach a suitable medium—in this case the fæces of a frog or toad.

When the contents of the large intestine of a frog or toad is examined, as a rule no monads of this species can be discovered. After a long and careful search a few cysts are usually—though not always—found to be present. Cysts are practically always present, probably, although they cannot always be found. For if some of the fæcal matter be placed in a carefully cleaned watch-glass and diluted (see p. 77) a favourable medium for the development of the cysts is formed. In the course of a few days—probably in about three days—the first monads will be found. When first liberated they differ from the adults in their small size, globular shape, and simple structure. They possess no reservoir or cytopharynx, and no food bodies. The protoplasm is peculiarly pale and transparent, not at all like the granular protoplasm of the adult. These monads develop gradually into the adult form, and then begin to divide in the usual way.

I have not been able to watch the liberation of the monad from the cyst, in spite of repeated efforts to do so. I think it highly probable that the animal is set free by the cyst dissolving, not by its bursting; for I have never seen any signs

of ruptured cysts at this time which would suggest that a monad had emerged. All the cysts do not dissolve at the same time. I have sometimes, after carefully watching cysts on a slide which contained no free monads, found monads just liberated on another part of the slide at the end of the observation. After the cyst has dissolved the flagellum probably grows up from the basal granule, but this again I have not been able to watch.

The cysts must reach the frog's rectum by entering at the mouth and traversing the alimentary canal. They are probably ingested by the frog with its food, or in water, and must be very widely disseminated in nature, as they appear to be present in practically all frogs and toads.

A similar kind of life is led by the shelled rhizopod Chlamydophrys stercorea, Cienk., whose life-history has been elucidated by Schaudinn (**44**). It lives in the fæces of various animals, undergoing a remarkable development there. It is to be found sometimes in the fæces of frogs and toads, living side by side with Copromonas. Its presence here has not been previously recorded, I believe.

Durable cysts of considerable thickness are formed by this organism, and it was proved by Schaudinn that it was necessary for these cysts to traverse the alimentary canal before they could develop in the excreta. Simply placing the cysts in the fæces does not suffice to open them. In Copromonas, however, this is not the case. As long as the cysts are allowed to remain in the fæces for a day or two they can undergo development. It is not necessary to pass through the frog. This can be shown in the following manner: A small drop of an old culture which contains many cysts is allowed to dry on a coverslip. This kills any free monads which may be present, but does not injure the cysts. The contents of the large intestine of a frog are taken and diluted with water or salt-solution. This is then boiled for some minutes, in order to kill any organisms which may be present, filtered, and boiled again. The resulting liquid is quite free from monads, but forms an excellent culture medium for

them. A drop of this is placed on the dried-up cysts on the coverslip, and a hanging-drop preparation made on a hollow-ground slide. The coverslip is carefully waxed round the edges, and examined from time to time. In three or four days monads are usually to be found swimming about and dividing. In preparations made in this way a large number of cysts never develop, and many perish by bacterial invasion. The first monads to appear are similar to those which first appear in the ordinary course in the fæces.

Occasionally the cysts open in the rectum of the frog, instead of waiting until they leave it in the fæces. In this case the monads become parasitic for a period. Division may take place inside the frog under such conditions, but I have never seen any signs of conjugation in this situation. This is due, perhaps, to the inhibitory effect of the numerous other Protozoa which live in the frog's intestine. Most of these die in the course of a day or two after removal from the frog.

Degeneration.

Even in my most healthy cultures a number of monads always underwent degenerative changes and died. Degeneration was also brought about by keeping the monads for many days in hanging-drop preparations. A want of oxygen has probably much to do with this, for much less degeneration occurs in the Schultze chambers—where oxygen is supplied by the presence of green algal filaments—during an equal length of time.

Degenerating monads sometimes lose their regular contours, and become irregular and lumpy looking. Owing to the stoutness of the cuticular covering they do not become amœboid, as is so often the case in degenerating flagellates. Not uncommonly individuals grow abnormally large, being apparently unable to divide when they reach a certain size. These large and irregular forms present a very different appearance from that of the ordinary individuals.

One of the first signs of degeneration is the vacuolation of

the cytoplasm. Large clear vacuoles appear at any point in the creature's body, and the reservoir often reaches enormous dimensions—sometimes completely filling the anterior half of the animal.

The nucleus increases in size at first, but later breaks up into small fragments of various sizes. The cytopharynx appears to dissolve. As the animal dies it comes to rest, and the flagellum ceases to move. If it be watched at this stage it is seen to gradually fade away—apparently dissolving.

The dead monad is permeable to bacteria at only one spot—the cytostome. Through this bacterial invasion comes. First, the minute bacilli enter, but later, as the breach is widened, hordes of large bacilli and spirilla force an entry. At this stage the corpse is a mere bag containing a seething mass of microbes. As the nutritive remains of the monad get exhausted the bacteria gradually forsake their prey, until finally nothing but the skeleton of the monad—consisting of the cuticle—is left. The cuticle is thick and very resistant. It persists for a very long time in the form of an open sack, the opening marking the site of the former cell-mouth.

It is worthy of note that one can often observe degenerating monads side by side in the same drop of fluid with perfectly healthy individuals in active division or conjugation. The bacteria are usually most dense during the second day and thereabouts, but degenerating monads are not usually seen until several days later, when the number of bacteria has very greatly decreased.

General Discussion.

(1) Nucleus, Flagellum, and Basal Granule.

I propose to say in the following pages a few words about some of the more interesting points which a study of the morphology and life-cycle of Copromonas raises. In the first place, I must say something about the morphology of

the nucleus, flagellum, and basal granule—three structures which are probably connected phylogenetically.

Prowazek (**36**) distinguishes four different types of nucleus among the Flagellata :

(1) Simple nuclei, with an evenly-distributed chromatic network, and no internal structures (karyosome, division centre, etc.), e.g. Herpetomonas.

(2) Vesicular nuclei, with direct division; with central chromatin mass surrounded by a clear zone, across which a more or less distinct network extends outwards to the nuclear membrane. Such a nucleus may be seen in some species of Bodo, and is well seen in Copromonas.

(3) Centro-nuclei[1] containing a "neucleolo-centrosome" (Keuten) and separate chromatin masses. This type of nucleus is characteristic of Euglena and its allies.

(4) Vesicular nuclei with karyokinetic division: e.g. Polytoma, Chlamydomonas, etc.

To these four categories we may make the addition of a fifth, for the reception of the kind of nucleus found in Tetramitus (Calkins [**7**]).

(5) Nuclei in which the achromatic division-centre lies freely in the cell, whilst the chromatin is diffuse in the form of chromidia.

The nuclei will be seen to be of very different structure throughout the group. This difference is no less marked in the method of division. All stages, from amitotic constriction into two to karyokinesis with chromosomes, achromatic spindle and division-centres are to be met.

Fisch (**19**) described mitosis in Codosiga in 1885. In 1894 Blochmann recorded its occurrence in Polytoma and Monas vivipara (**2**) and in Euglena (**3**). His pupil Keuten carefully worked out the nuclear division in this last form in 1895 (**25**), describing the nucleolo-centrosome and a longitudinal splitting of the chromosomes. Schaudinn next year

[1] The centronucleus, as defined by Boveri, is a nucleus which contains a cytocentre—either in a consolidated or diffuse form. In the case of Euglena, etc., the cytocentre is the neucleolo-centrosome, i.e. is of the consolidated type.

(1896) brought forward some interesting observations on Oxyrrhis (**42**). He discovered that the intra-nuclear division-centre—the nucleolo-centrosome—could be made to leave the nucleus and enter the cytoplasm by placing the animal in diluted sea-water. In the cytoplasm the division-centre might grow in size and even divide. These observations are of great interest when considered in relation to the nuclear origin of the centrosome in Actinosphærium and Acanthocystis, as demonstrated by Hertwig and Schaudinn.

Mitosis was demonstrated in various Chlamydomonadina by Dangeard in 1898 (**15**). In the same year Calkins' account (**7**) of the remarkable nuclear phenomena in Tetramitus appeared. In this animal the chromatic and achromatic parts of the nucleus are completely separate, except during division. The former is in the form of chromidia, the latter in the form of a large consolidated division-centre. Prowazek (**35**) and Dangeard (**16**) again worked out mitotic division in Polytoma in 1901. The former described the presence of about eight chromosomes, the latter found not more than six. Prowazek also described the formation from the nucleus of a minute body—the "entosome"—which appears to act as a division-centre. Various euglenoids were investigated by Dangeard (**17**) in the following year, and one (Entosiphon) was described by Prowazek (**37**). It appears to have a method of division similar to Euglena, but more primitive. Dangeard (**18**) also described mitosis in Monas vulgaris. In 1904 Steuer gave us a description of mitosis in the euglenoid Eutreptia (**48**), and Awerinzew has just described anew (**1**) the nucleus and its division in Chilomonas.

Amitosis is found in some forms. It was recorded in Monas guttula by Prowazek (1903 [**36**]), and by the same writer in Bodo lacertæ in 1904 (**39**). From his description of the division—apparently amitotic—of the nucleus of Trichomastix it would seem that the axial rod plays the part of a directive centre.

The various kinds of division, whose history I have just

imperfectly sketched, can all be brought into a more or less perfect series with one another. Beginning with Copromonas, we have a nucleus in which chromatic and achromatic elements are united in the resting nucleus, and do not separate at division. A step further we find the nucleus of Entosiphon, which, although apparently identical with that of Copromonas when in the resting state, shows some separation of achromatic spindle and chromosomes during division. In the Chlamydomonadina also a similar condition is found, but here the chromosomes can be clearly counted. A stage further we come to the nucleus of Euglena, in which achromatic division-centre and chromatic elements are distinct from one another even in the resting nucleus. In Eutreptia the chromatin seems, in the resting state, to be less closely aggregated round the division-centre, and the outline of the nucleus is irregular. Chilomonas (see Awerinzew [1]), shows a nucleus (consisting of the neucleolo-centrosome and part of the chromatin?) surrounded by chromidia. Such a condition may be derived from that seen in Eutreptia by the loss of the nuclear membrane. Finally, we reach the nucleus of Tetramitus, in which all the chromatin has left the division-centre and lies freely in the cytoplasm in the form of chromidia.

That this is a progressive phylogenetic series I do not believe. I think it probable that the Copromonas type of nucleus is the most primitive, and the Euglena type the most highly evolved. From the Euglena type the Tetramitus type may have arisen by regressive changes. I mean that I believe the inter-relationships should be expressed in the form of a V with Euglena at the angle and Copromonas and Tetramitus at the ends rather than as a straight line. These points will have to be taken into account when the Flagellata come to be re-classified.

Let us now leave the nucleus and consider the flagellum and its connections. The flagellum has been classified under three headings by Prowazek (36), according to its relations to the nucleus. These three classes are :

(1) Flagella arising directly from the nucleus, as in Mastigamœba, etc.

(2) Flagella united to the nucleus by means of an intermediate link, the "zygoplast"—e.g. species of Monas, Chlamydomonadina, etc.

(3) Flagella arising from a basal granule, independent of the nucleus, a condition seen in Copromonas.

And to complete the list I will add:

(4) Flagella arising from a special nucleus—the kinetonucleus—as in the trypanosomes.

An intermediate condition between (2) and (4) may be seen in Hæmoproteus noctuæ, where kinetonucleus and trophonucleus remain united by an achromatic thread. Many varieties of (2) are to be seen. In Chlamydomonas, for example, the flagella are attached to a basal granule from which a strand—the rhizoplast—runs to the nucleus, on the membrane of which it ends in a knot-like enlargement.

The first arrangement occurs also in the swarm-cells of Mycetozoa (Plenge [**32**]). It was first described in Mastigamœba aspera by F. E. Schultze (**46**) in 1875, and has since been observed in allied forms by various writers (cf. Goldschmidt [**22**]). It may be compared with the attachment of the axopodial rays to the nucleus in Heliozoa—e.g. in Camptonema (Schaudinn [**41**]), also with the axial rod and nucleus of Trichomastix (Prowazek [**39**]). Plenge has suggested that the flagellum in soft-bodied organisms like Mastigamœba is attached to the nucleus because it happens to be a relatively fixed point.

The homology of the basal granule still remains obscure. It has been thought to be homologous with the basal granule (end knob) of the tail filament of a spermatozoon. And the end knob has been shown from the work of Moore, Meves, Paulmier, Hermann, Lenhossék, Benda, Korff, and many others to be the centrosome.[1] The axial filament is an outgrowth of it.

[1] It is beyond the scope of the present paper to enter in detail into the enormous literature on this aspect of the centrosome. The reader who seeks further information on the subject will find an excellent account in Maier (**30**), and also much that is of importance in Wilson (**53**).

This led to the formulation of the well-known Lenhossék-Henneguy hypothesis, which states that the centrosomes form the basal granules of flagella and cilia. The work of Gurwitsch, Maier, Henry and others on ciliated epithelium and infusoria clearly shows that this generalisation cannot be made. The flagella certainly arise from centrosomes in sperms and in the so-called "Centralgeissel" arrangement, but not as a rule in other cases. The basal granules of flagella and cilia appear to be merely cytoplasmic thickenings in most cases (Henry, Gurwitsch, etc.).

The possible homology of the basal granule with the trypanosome blepharoplast[1] must not be forgotten. For a long time the nature of this structure remained doubtful. Wasielewsky and Senn regarded it as an ectoplasmic thickening, a kinoplasmic differentiation unrelated to the nucleus. Rabinowitsch and Kempner thought it was a nucleolus. And whilst Laveran and Mesnil regarded it, from a Lenhossék-Henneguy point of view, as a centrosome, Bradford and Plimmer compared it with the infusorian micronucleus. Its real nature was revealed by Schaudinn's study of Hæmoproteus noctuæ (**43**). He showed that it was a separate nucleus specially concerned with the locomotory functions of the cell, a kinetonucleus, taking origin from the original compound nucleus. The origin of the blepharoplast from the synkaryon has since been observed by Bradford and Plimmer in Trypanosoma brucii.

From the kinetonucleus the flagellum takes origin. It is, however, a true nucleus, undergoing reduction and fusion at conjugation. A similar reduction, etc., occurs in Herpetomonas, according to Prowazek (**38**).

My observations on the basal granule of Copromonas and the part it plays in division (see p. 90) suggest that it may indeed be homologous with the kinetonucleus. An interesting comparison may be made with the flagellar apparatus in some Rhizomastigina. In Mastigamœba Prowazek (**36**) states that the flagellum is retracted into the nucleus before

[1] For the more important literature see Woodcock (**54**).

this divides. Presumably new flagella subsequently grow from the daughter-nuclei, as from the basal granules of Copromonas. A similar condition appears to exist in Mastigina, according to a recent paper by Goldschmidt (**22**). In some Bodos the basal body is as large as the nucleus, and stains very strongly with nuclear stains. It divides in division of the monad.

Unfortunately, although many interesting comparisons can be made, the problem of the homologies of these various structures must still be left unsolved. It can be settled by further research only. At present it is not possible to make any definite pronouncement on the subject.

Conjugation.

Much controversy has taken place on the subject of conjugation in the Flagellata. Only a few years ago it was denied that any conjugation existed in the group. But now, owing largely to the labours of Prowazek, it can be definitely stated that conjugation occurs here as in all the other chief subdivisions of the Protozoa.

Early observers—Cienkowski, Stein, and others—described fusions between two monads in various species, interpreting them as processes of conjugation. In the years 1873 to 1875 the remarkable observations of Dallinger and Drysdale (**12**) appeared, and gave rise to much dispute. Processes which, if they really exist, must be described as conjugation, were recorded in many monads, which have subsequently been referred to the genera Polytoma, Bodo, Monas, Cercomonas, etc. Following conjugation, some remarkable methods of sporulation, including the formation of ultramicroscopic "microspores," were claimed to have been seen. Saville Kent (**24**) accepted the work of these two observers, but Bütschli (**6**) regarded it with great scepticism, admitting that conjugation takes place in the Phytomonadina only. A similar attitude was adopted by Klebs (**28**) in 1892, and by Blochmann (**4**) three years later. In the latest systematic

account of the Flagellata which has appeared (**47**), Senn (1900) regards conjugation as still unproven in the group.

Apart from the Phytomonadina all accurate knowledge of the matter has been acquired during the last half-dozen years. The most important researches are those of Prowazek and Schaudinn, the latter throwing much light upon the trypanosomes and their allies. In the Phytomonadina conjugation has been demonstrated in many genera: e. g. in Polytoma (by Krassilstschik [**29**], Dangeard [**16**], Prowazek [**36**], and others); in Chlamydomonas, etc., by Dangeard (**14**, **15**); in Dunaliella (Teodoresco [**50**]), Volvox, Pandorina, and others.

Goldschmidt (**22**) has just worked out a complete life-history in the Rhizomastigina—in Mastigella. It would appear from his account that the mastigamœbæ are closely allied to the Rhizopoda, for he describes karyokinesis, formation of gamete-nuclei from chromidia, differentiated micro- and macro-gametes, etc., which strongly recall the conditions seen in rhizopods.[1]

Apart from these two groups—the Phytomonadina and Rhizomastigina—conjugation has been shown to occur in Monas vivipara (**36**) and Bodo lacertæ (**39**) among the Protomonadina, and in the following members of the Polymastigina: Trichomonas intestinalis (**44**, **39**), Hexamitus intestinalis (**39**), Lamblia intestinalis (**44**). It has also been proved to take place in Trypanosoma lewisi, Hæmoproteus (Halteridium, Trypanosoma) noctuæ (**43**), and in Herpetomonas muscæ-domesticæ (**38**). In all these forms heterogamy is found. But autogamy takes place in some flagellates, e. g. in Trichomastix lacertæ (**39**), Herpetomonas muscæ-domesticæ (**38**), Bodo lacertæ (**39**), etc. Sexual differences between the

[1] Perhaps Pseudospora should be considered in the same category. It certainly shows affinities with the Rhizopoda, but from Miss Robertson's recent paper, 'Quart. Journ. Micros. Sci.,' vol. 49, 1906, p. 213, it is not clear to me how far gamete-formation in this animal corresponds with that of the Mastigamœbæ.

conjugants exist in a few forms—in trypanosomes and their allies, and in Bodo lacertæ.

Conjugation has not been proved to occur in the Chromomonadina, and no proof of its occurrence in the Euglenoidina has been given before. Indeed, Copromonas is the only uniflagellate monad—with the doubtful exception of the "uniflagellate monad" of Dallinger and Drysdale, the "Monas dallingeri" of Kent—which has had its life-history worked out. And even in this I cannot claim that it has been done with completeness. There can be little doubt that there is still much more to be discovered of the ways of life of Euglena and its allies. The observations of Entz, Zacharias, or others have certainly not proved that conjugation takes place in this order.

Nuclear Reduction.

A reduction of the nuclear chromatin is a phenomenon which is usually met with in connection with conjugation. Leaving the Phytomonadina out of the question for the moment, it may be stated that nuclear reduction occurs in all flagellates as a preliminary to karyogamy. It has been observed in every case where accurate investigation has been made.

The most common method by which this is affected is by two divisions of the gamete nucleus, resulting in the formation of two reduction nuclei ("polar bodies," "Richtungskörper") and a reduced gamete nucleus. The reduction nuclei degenerate, and the reduced nuclei approach one another and fuse. Such phenomena occur in Trichomonas intestinalis (**39, 44**), in Bodo lacertæ (**39**), in Hexamitus intestinalis (**39**) and in the autogamy of Trichomastix lacertæ (**39**). Reduction divisions of both trophonucleus and kinetonucleus are found in trypanosomes and allied forms. And, as I have already shown, a nuclear reduction consisting probably in two reduction divisions (see p. 97) takes place in Copromonas. In Monas vivipara reduction appears to be brought about by the expulsion of chromidia from the nucleus (**36**).

In spite of all the attention which has been bestowed upon the Phytomonadina, no one has yet succeeded in demonstrating that a nuclear reduction takes place in the gametes before conjugation. Even in Polytoma, which has received the attention of so many investigators, no division of the gamete nuclei before fusion has been seen (cf. **16**, **21**, **29**, **36**). Prowazek [**36**] suggests that the formation of dwarfs ("Zwergindividuen") may have some significance in this respect. In Chlamydomonas also, Dangeard [**14**] finds that no nuclear reduction takes place before conjugation, the conjugants having the same number of chromosomes as the ordinary individuals. He suggests that reduction occurs during germination.

Now in the lower plants reduction does not seem always to occur just before conjugation. For instance, it occurs at the beginning, not at the end, of the life-cycle in the Desmidiaceæ, Spirogyra, etc., with the first division of the fertilised oosphere. That a similar condition obtains in Polytoma is possibly indicated by the fact (observed first by Krassilstschik, and since confirmed by Prowazek) that the first division after leaving the cyst is into eight, whilst subsequent divisions are into four daughter-cells.

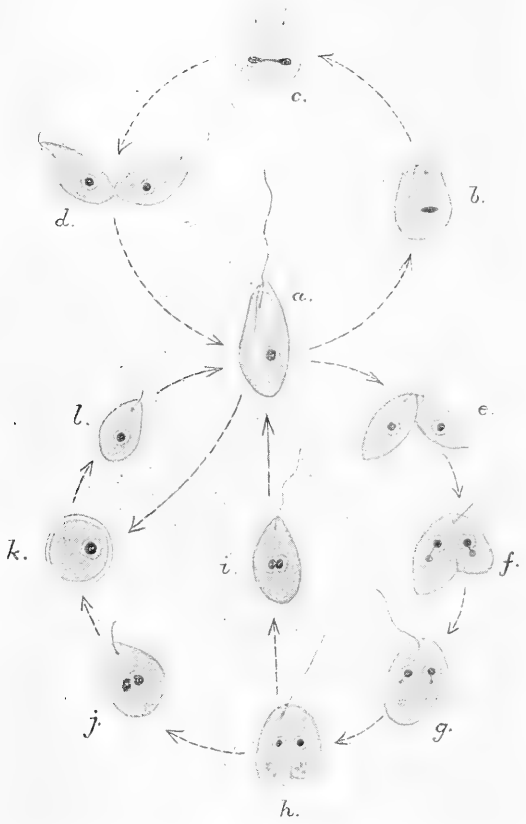
I believe the foregoing facts furnish further evidence of the plant affinities of the Phytomonadina, and consequently for their sharp demarcation from the rest of the Flagellata.

Summary of Life-cycle of Copromonas subtilis.

In conclusion I will give a brief synopsis of the life-cycle of Copromonas. This can be most easily done by means of a diagram (see text-fig. C). Starting with the adult monad, *a*, it will be seen that the life-history comprises two different cycles—an asexual (upper cycle, figs. *b*–*d*), and a sexual (lower cycle, figs. *e*–*l*). This latter is intimately connected with the formation of resting cysts. During the asexual cycle multiplication takes place by means of longitudinal division. The organism comes to rest, and the flagellum is drawn in.

The nucleus divides, as does the basal granule of the flagellum. Two new flagella are formed, and two new cytostomes. One of the daughter individuals takes the contractile vacuole, the

TEXT-FIG. C.



other develops a new one. The reservoir is halved, each daughter-cell taking half. The nucleus divides amitotically (figs. *b*, *c*, *d*).

After a time the monads conjugate in pairs (*e*). The

anterior ends come in contact and fuse. One flagellum is lost. By means of the remaining one the conjugants swim about during conjugation. Each nucleus divides once and one of the daughter-nuclei at once degenerates (*f*). A second nuclear reduction occurs in which a chromatin granule (or granules?) is extruded (*g*). This process might also be described as a heteropole division. Two courses are now open to the partially fused monads containing two reduced nuclei: (1) The nuclei may fuse (*i*) and the cell undergo a process of remodelling, whereby a zygote monad—indistinguishable from an ordinary individual—is formed (*a*); the zygote may then continue to divide (*a–d*), and subsequently its descendants may encyst (*k*). (2) Or the flagellum is drawn in, the zygote rounds itself off (*j*), and a cyst is formed. This is at first soft and gelatinous, but subsequently becomes hardened and more or less encrusted. The nuclei fuse inside the cyst. In whichever way the cysts have been formed they are apparently identical in appearance and subsequent history. They are capable of being dried up, and when they reach a suitable medium once more (i.e. the fæces of the frog) they liberate a small hyaline monad (*l*) which grows up and repeats the cycle of events just described.

The gametes are not differentiated from the ordinary individuals—i.e. every individual appears to be a potential gamete—and no sexual differences exist between the two members of a pair of conjugants. It may be noted, however, that one conjugant appears to absorb the other after it has lost its flagellum.

The cysts are swallowed by frogs and toads, and reach the rectum by way of the digestive tract. As a rule the cysts do not set free their contents until the fæces have left the frog. But sometimes the monads emerge from their cysts and lead a semi-parasitic life in the large intestine. Development does not appear ever to be completed inside the frog.

Note on Copromonas sp., from the Fæces of the Newt.

In searching through the intestinal contents of the common newt (Molge vulgaris) I came across a few small monads very clearly resembling C. subtilis. I was therefore led to make some culture experiments with the fæces, in the manner adopted in the case of frogs and toads. As a result I found that a very similar organism exists in this situation, only differing from C. subtilis in its smaller size. It is possible, indeed, that it is the same species, which does not attain its full size in the fæces of the newt.

The size of this monad is approximately 7 μ–10 μ in length by 3 μ–4 μ in breadth. It reproduces in the fæces by longitudinal division in exactly the same manner as C. subtilis. Conjugation and encystment have never been observed.

In view of the fact that so little of the life-history has been investigated, I think it best not to bestow a specific name upon this animal. Nevertheless, I believe it will be found to be specifically distinct.

A figure of the organism is given on Pl. 5 (fig. 48).

Zoological Laboratory, Cambridge,
August, 1907.

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DESCRIPTION OF PLATES 4 & 5.

Illustrating Mr. C. Clifford Dobell's paper on "The Structure and Life-History of Copromonas subtilis, nov. gen. et nov. spec.: a Contribution to our Knowledge of the Flagellata."

PLATE 4.

Figs. 1–5 and 11–20 are drawn from living specimens, under a 2·5 mm. apochromatic water-immersion, with compensating ocular 12. The remainder are from permanent preparations—fixed hot sublimate-alcohol, and stained

Heidenhain's iron hæmatoxylin; drawn under a 3 mm. apochromatic oil-immersion, compensating ocular 18.

Fig. 1.—Copromonas subtilis—adult individual before division.

Fig. 2.—Same individual, drawing in flagellum preparatory to division.

Fig. 3.—The same; flagellum completely drawn in, and nucleus dividing.

Fig. 4.—Still later; two new flagella growing up side by side, nucleus in later stage of division, etc.

Fig. 5.—Later; nucleus and reservoir divided; growth of new flagella, etc.

Fig. 6.—Stained preparation, showing chief anatomical features.

Figs. 7–10.—Various consecutive stages in division.

Figs. 11–16.—Conjugation.

Fig. 11.—Attachment of two monads by their anterior ends.

Fig. 12.—The same, twenty-five minutes later. One flagellum has been drawn in.

Fig. 13.—The same, thirty minutes later.

Fig. 14.—Fifteen minutes later.

Fig. 15.—Twenty minutes later; monads almost completely fused.

Fig. 16.—Fifteen minutes later. Complete fusion has taken place, and the resulting zygote has become re-modelled to the form of an ordinary individual.

Figs. 17–19.—Various forms of cyst met with in cultures.

Fig. 20.—Monad shortly after liberation from cyst.

Figs. 21–33.—Conjugation and encystment.

Fig. 21.—Fusion of two individuals; one flagellum is being drawn in.

Fig. 22.—Further fusion has taken place; only one flagellum present.

Fig. 23.—First nuclear reduction. On the left, nucleus in an early stage of division (cf., Plate 5, fig. 35); on the right, nuclei almost divided.

Fig. 24.—The reduction nuclei have been formed, and are degenerating. They are pale and situated below the gamete nuclei. (This specimen is slightly flattened.)

Fig. 25.—Further nuclear reduction by heteropole division (granule extrusion). Parts of the first reduction nuclei are seen degenerating below.

Fig. 26.—Later stage, containing two reduced gamete nuclei.

Fig. 27.—Nuclei fusing; zygote assuming motile monad form.

Figs. 28–30.—Fusion of nuclei and encystment.

Fig. 31.—Descendant of zygote monad before encystment.

Fig. 32.—A similar organism at a later stage.

Fig. 33.—Resting cyst; formed from either 30 or 32.

PLATE 5.

All drawings are made from permanent preparations, stained Heidenhain's iron hæmatoxylin. Fixation: Fig. 41, osmic vapour; remainder, sublimate alcohol. Figs. 34–40 drawn under a 2 mm. apochromatic oil-immersion (apert. 1·40), compensating ocular 12. The others under the 3 mm. apochromatic, compensating ocular 18.

Figs. 34–40.—Nuclear division.

Fig. 34.—Nucleus before division.

Fig. 35.—First stage in division; somewhat fusiform; chromatin granules extruded at opposite poles.

Fig. 36.—Nucleus now in the form of a short rod.

Fig. 37.—Nucleus dumb-bell shaped.

Fig. 38.—Chromatin aggregating at the ends of the dumb-bell, so as to give rise to two daughter-nuclei connected by a broad band.

Fig. 39.—Daughter-nuclei still connected by a filament.

Fig. 40.—Two fully-formed daughter-nuclei.

Figs. 41–46.—Flagellum and its method of multiplication. The drawings are of the anterior end of the organism.

Fig. 41.—Ordinary individual before division, showing basal granule, etc.

Fig. 42.—Flagellum being drawn in.

Fig. 43.—Flagellum completely drawn in, only basal granule left.

Fig. 44.—Division of basal granule. The daughter granules lie one on either side of the reservoir, and are still connected.

Fig. 45.—Growth of new flagella from the basal granules. The latter are still seen to be connected by a fine filament.

Fig. 46.—Later stage. The flagella are longer, and are seen growing out of the depressions caused by the formation of the new cell-mouths. The reservoir is cleft in two.

Fig. 47.—Abnormal union of three monads.

Fig. 48.—Copromonas sp. from the fæces of the newt.

Notes on some Parasitic Protists.

By

C. Clifford Dobell, B.A.,
Scholar of Trinity College, Cambridge.

With Plate 6.

During the last few years the Bacteria and their allies have engaged the attention of so many workers, have caused so much controversy, and have proved to be of such great interest—phylogenetically and otherwise—that I believe any observations, however small, which would supplement our knowledge of their cytology or life-history, are much to be desired. With this conviction I offer these few imperfect notes on some of the more interesting protists which have come under my observation from time to time.

I. Bacillus flexilis, n. sp. (Pl. 6, figs. 1—19.)

More than five years have now elapsed since the appearance of Schaudinn's epoch-making paper on Bacillus bütschlii, a parasite of the common cockroach, P. orientalis. Yet, apart from Schaudinn's own further researches on the Bacteria, no similar life-history has since been recorded. However, I can now record the existence of an organism which in many respects exhibits a remarkable similarity to B. bütschlii.

I propose to name this organism Bacillus flexilis, n. sp., although I must point out that I do not believe it should

really be placed in the genus Bacillus at all. But B. bütschlii has now remained in this genus for some time, and I think my organism must go where it goes. Both forms will probably have to be removed—perhaps, as Schaudinn suggested, to the genus Dispora, Kern. The large size of these organisms, their life-history and formation of two spores, all distinguish them from the ordinary Bacilli. The specific name is given on account of what is, at first sight, the most striking characteristic of the organism—namely, its flexibility.

B. flexilis is found in the large intestine of frogs and toads —Rana temporaria, L., and Bufo vulgaris, L. It is, like B. bütschlii, of rare occurrence; I have found it in only 3—4 per cent. of animals examined, and on only one occasion in large numbers. I have also found a similar, though longer and more slender organism, in the common newt.

In size the organism varies considerably, and may be any length from 15 μ to 35 μ. On one occasion I found a specimen (undivided) which had attained a length of 39·5 μ. An average length is about 25 μ. The breadth is from 2—3 μ. It will be seen, therefore, that, although a giant among Bacteria, this protist is only about half the size of B. bütschlii.

The shape is that of a long rod, with the ends bluntly pointed rather than rounded. The protoplasm is finely granular and somewhat alveolar in the living organism, but by no means so distinctly "honeycombed" as in B. bütschlii (see Pl. 6, fig. 1). A strongly-developed outer layer can be seen on careful focussing (fig. 1) consisting of the pellicle and perhaps also of an ectoplasmic sheath. The organism is clad all over with very fine cilia ("flagella" of the bacteriologist), which can be demonstrated in stained preparations only (see fig. 9).

A large number of granules inside the organism are found to stain with nuclear stains. I am inclined to regard these as being composed of chromatin, and the organism, therefore,

according to my view, contains a "diffuse nucleus" in the form of a chromidial system.[1]

The movements of B. flexilis are very characteristic. By means of its "flagella" it swims rapidly from place to place in either direction. In addition to these locomotory movements, intrinsic movements can be observed. They consist, for the most part, in a lateral bending, and appear to be largely passive, determined by the disposition of the débris through which the organism is making its way. Owing to these movements, the organism presents a curved, S-shaped or sometimes somewhat spiral appearance. As it swims about its ends often oscillate slowly from side to side, pendulum-wise.

In being so remarkably supple, this Bacillus differs from all others. In fact, rigidity has sometimes been used as a criterion for judging the bacterial or protozoan nature of an

[1] It is perhaps necessary to say a few words about these granules in Bacteria. Colourable granules have been long known to occur in bacterial cells. They were described as "metachromic bodies" by Babès, "red granules" by Bütschli, "sporogenic granules" by Ernst, etc., etc. They have been variously interpreted: by some (Bütschli, Schewiakoff, etc.) as chromatin, by others (Fischer, Migula, etc.) as metabolic products, by others (Meyer, Grimme, etc.) as "volutine," by others still (e.g. Podwyssotzski) as products of degeneration, by others again (e.g. von Behring) as toxigen granules, and so on and so on. All views have, in consequence, been maintained regarding the cytology of Bacteria—from that which regards them as all cytoplasm to that which regards them as all nucleus. The latter view has recently been upheld by Růžička, who believes Bacteria to consist entirely of nuclein, on account of their resistance to artificial gastric juice. Bütschli first put forward this view, and it was held by Klebs, Hüppe, and many others subsequently. On the other hand, many writers, since Schottelius in 1888, maintain that a true nucleus exists in Bacteria. Such a view has been held by Vejdowský, Mencl, A. Meyer, etc., etc., whose work seems to prove conclusively that, in some cases at least, a distinct nucleus is present.

It appears highly probable that more than one kind of granule exists in the bacterial cell, and that in many cases the granules are in part chromatin. Schaudinn, Guilliermond, and others adopt this view. The "metachromic bodies" are probably reserve material. Meyer and Guilliermond have shown that they exist side by side with a true nucleus in the Ascomycetes—an observation of much interest.

organism—for instance, in the case of the spirilla and spirochæts. In speaking of Bacillus bütschlii, Schaudinn says, "Biegung des Stäbchens findet man äusserst selten;" so that in this respect it must differ markedly from my Bacillus. The only other organisms which appear to be in any way comparable, are the remarkable forms included in the Myxobacteriaceæ of Thaxter. But here, although the rods are flexible, they are not motile in the same manner, for they progress by creeping movements on a resistant surface. (See Baur's description.)

Division is effected in B. flexilis by a simple constriction into two, as in B. sporonema, Schaudinn. In the method of division, therefore, another point of difference between my organism and B. bütschlii is seen: for in the latter, division takes place by septation. I have watched division in the living organism, and have stained permanent preparations at various stages, but I think it is not necessary to enlarge upon a process which has been already described in so many bacteria (see Plate 6, fig. 10).

The most interesting phenomena are those connected with spore formation. I will describe these first, and comment on them afterwards. Unfortunately, I have not been able to follow the whole process from beginning to end in one and the same living organism: for, at the end of a period varying from three to five hours, my protists always died in hanging drop or coverslip preparations. But I have been able to watch spore-formation in different organisms in consecutive stages, and I believe, the following is a substantially correct account of the normal course of events. The observations have all been confirmed on stained material.

The organisms which are about to form spores are of considerable length—usually about 30 μ to 35 μ—and are filled with distinct granules, which stain a purple-red with Giemsa. After swimming about in this condition for some time, the rod begins to show a slight constriction at its middle, and appears to be about to divide (fig. 10). Division is not completed, however, and after the lapse of some time, it can be

seen that the constriction, which at one period almost severed the rod into two daughter rods, is slowly disappearing (fig. 11). The granules at this period are very distinct and numerous, but I have never succeeded in convincing myself that any streaming movements were taking place, on account of the active movements of the organism. It is probable, however, that such granule-streaming does occur, as in B. bütschlii, where Schaudinn was able to watch it with great precision. As the constriction disappears, the granules begin to rearrange themselves in the cell, but so slowly that their movements cannot be noticed whilst the organism as a whole remains motile. At the end of some hours, the granules are seen to be disposed in a somewhat irregular spiral (figs. 2 and 13). This spiral is quite distinctly visible in the living organism. It reminds one of the figures given by Swellengrebel of B. maximus buccalis. As in this case, the spiral appears to be formed of chromatin, the chromidia having rearranged themselves in this form.

I do not know how long the organism remains in this condition, but it is probably for several hours. At the end of this time, small, clear areas can be seen at either end of the spiral (fig. 4). These are the spore rudiments, and at first they look like vacuoles. It can be seen in the stained preparations that they are formed by the concentration of the chromatin at these spots (fig. 14). These chromatic areas increase in size (figs. 5, 15), and as they do so the intermediate part of the spiral begins to break down (figs. 14, 15). When a later stage is reached, the spores appear as dense, ovoid masses of chromatin, and the intermediate chromatin breaks up into fine granules (fig. 16). After this, a clear area makes its appearance round the spore (fig. 6). This is the first appearance of the spore membrane. Its presence is easily seen in the living organism, and is strikingly displayed in Giemsa preparations: for the spores, which have hitherto been coloured red, now appear deep blue (fig. 17). The spore membrane gradually grows hard and resistant, and as its induration increases, it stains less and less (fig. 18). Finally,

the spores become exceedingly hard and refringent (fig. 7) and refuse to stain in the slightest degree.

Whilst the spore membrane has been forming, the intermediate part of the spiral has broken down completely, and as it degenerates, the protoplasm changes its staining reactions. At the beginning, the spiral was red, in a bluish cytoplasm (figs. 13, 14, 16). As it degenerates, and diffuses through the protoplasm, it imparts to it a purplish (fig. 17), and finally a pink tinge (fig. 18). The spores, which at first occupied the ends of the cell, now move towards one another somewhat, so that little polar caps of protoplasm are left at the extremities of the rod. In contradistinction to the rest of the protoplasm, these caps stain blue with Giemsa (fig. 18). The rods with their two terminal spores present a very characteristic appearance at this stage (figs. 3, 18), for they remain actively motile for some time longer, sometimes for several hours. In the end, they come to rest, and the protoplasm degenerates (fig. 19), so that the two fully-formed spores alone remain, connected by the dead and broken remains of the organism.

The fully formed spores, like those of B. bütschlii, vary somewhat in shape, and show a small cap-like eminence at one end (fig. 8). This, in B. bütschlii, is the pole at which germination occurs. I regret that I have not been able to watch the germination of the spores in B. flexilis. In size, the spores are about 5 $\mu \times 2\ \mu$, and hence cannot be easily mistaken for any other spores found in the same locality.

I should point out that the figures of stained specimens have all been drawn from Giemsa preparations in which drying has taken place between staining and mounting. They are all, therefore, somewhat flattened out, and so appear broader than they really are (see especially figs. 9, 14, and 17). This flattening of the organisms serves, however, to show their structure more clearly. And although the method is not to be recommended for the most accurate work, it gives very pretty results. I have used the method only in conjunction with others (see Appendix), but the results have

been essentially the same in all cases, and merely confirm what can be seen in the living organism.

I have encountered very many degenerate and plasmolysed forms of different kinds. Almost all of these have been nearly identical with similar conditions figured by Schaudinn in B. bütschlii. Indeed, his figures might easily stand for B. flexilis. I have found vacuolated forms, forms in which abnormal division into three is occurring, forms in which an abnormal formation of a single spore has taken or is taking place, etc., etc. I do not think it is worth while to describe these again in detail, but I may mention that I have also found involution forms which reached the enormous length of nearly 200 μ, incompletely divided into as many as six cells, the whole chain writhing fitfully for hours, but owing to its unwieldy length unable to make any progress.

Spore-formation in Bacillus flexilis resembles in a remarkable manner that of Bacillus bütschlii, as anyone who will take the trouble to compare my figures with those of Schaudinn can see. In both organisms the incomplete division into two daughter-cells is only temporary; it is followed by a re-arrangement of the granules into a spiral filament, and by the heaping up of the granules at the ends, two spores arise in a closely similar manner—a part of the spiral degenerating.

Schaudinn, who discovered this remarkable process, looked upon it as a kind of primitive conjugation between daughter-cells, comparable to that which occurs in Actinosphærium (R. Hertwig), and—as he himself showed later—in Entamœba coli. He was also able to demonstrate the existence of a similar phenomenon in Bacillus sporonema, Schaudinn. I think the evidence is strongly in favour of such an interpretation.

A striking analogy exists in the case of the yeasts, which have yielded such interesting results to recent investigators. It is now conclusively proved that in some genera—e. g. Schizosaccharomyces and Zygosaccharomyces—conjugation, followed by sporulation, takes place between

daughter-cells which have not separated. These observations were made quite independently by Barker, Guilliermond, and others. A comparable process is also to be seen in the Algæ—e. g. Spirogyra—where adjacent cells may sometimes conjugate and form a zygospore. And there are further analogies to be found in the autogamy of the Protozoa (Bodo, Trichomastix, etc.), and the curious form of parthenogenesis sometimes seen in the Metazoa—for instance, in Artemia, according to the well-known description of Brauer.

The breaking up of the intermediate portion of the spiral may be regarded as a nuclear reduction—though it has yet to be proved that the filament really consists of chromatin. Similar filaments, consisting very probably of chromatin, occur in allied organisms, for example, in B. maximus buccalis (Swellengrebel), in Spirochæta balbianii (Perrin), and in Spirillum giganteum (Swellengrebel) (cf. also B. spirogyra, infra).

II. Bacillus spirogyra, n. sp. (Pl. 6, figs. 20*a*, *b*, *c*.)

I have found this Bacillus on several occasions in the rectum of frogs and toads. In the living condition it is an actively motile rod, of high refractivity. It shows no internal structure until stained—a circumstance which I believe to be due chiefly to the great thickness of its pellicle. In length the organism measures about 8—11 μ, and in breadth about 1·5—2 μ. It is, therefore, a bacillus of considerable size.

Upon staining the organism some additional points in its morphology can be made out. The very thick pellicle stains pink with Giemsa, and inside the organism a spiral filament is seen, which is stained like chromatin. That is to say, it becomes red or purple with Giemsa, black with iron-hæmatoxylin, etc. I believe it to be of a nuclear nature. It is reminiscent of the spiral structure described by Swellengrebel in B. maximus buccalis (see Pl. 6, fig. 20*a*).

The method of division is by septation, as in B. bütschlii. Before division the nuclear spiral becomes more coiled, and

very distinct. In the middle of the cell which is about to divide a transverse septum makes its appearance, staining strongly with Giemsa or iron-hæmatoxylin. The ends of the organism also stain darkly at all stages of its life; this staining of the ends and of the septum being a common condition in bacteria. At this stage the organism has the appearance shown in fig. 20*b*. Later the septum splits into two, and its halves then give rise to the ends of the daughter-cells (fig. 20*c*). These then separate.

Although this Bacillus resembles B. maximus buccalis in possessing a spirally disposed nuclear filament, it differs from it in its method of division. For in the latter a longitudinal splitting of the filament appears to take place, whereas in the former, as I have just shown, a transverse division can be seen. Swellengrebel's very careful micro-chemical investigation of B. maximus buccalis renders it highly probable that the filament consists of a substance which, if not chromatin, is at least very closely allied to it. In my Bacillus I have seen no separation of "chromosomes" and achromatic substance during division.

As this Bacillus does not appear to have been described before, I have named it Bacillus spirogyra, n. sp., from the fanciful resemblance which it bears to a Spirogyra cell with its contained chloroplast.

I have not yet succeeded in tracing out the rest of its life-history.

III. Spirillum monospora, n. sp. (Pl. 6, figs. 21*a*, *b*, *c*, *d*.)

A large species of Spirillum is not unfrequently found in the large intestine of frogs and toads. As I can find no record of it in the work of other investigators, I have called it Sp. monospora, n. sp., from the fact that it forms a single large terminal spore.

This Spirillum is usually S-shaped. Its dimensions are ca. 6—8 μ × ca. 1·5 μ. It is actively motile, and can be seen (in deeply stained iron-hæmatoxylin preparations) to possess

a flagellum (or bunch of flagella?) at either end. No simple nucleus, such as Kunstler and Gineste have described in Sp. periplaneticum, K. and G., can be made out; nor can a nuclear spiral, like that which Swellengrebel observed in Sp. giganteum, be seen. The only contents which stain consist of a number of granules, which recall the figures of Sp. volutans given by Bütschli. In this case again I can merely say that these granules stain in a manner which causes me to believe that they are chromatin (see Pl. 6, fig. 21*a*).

Transverse division takes place in the manner usually seen in the Spirilla. It will not, therefore, be necessary to describe the process. I may say, however, that I have not been able to find any specimens in which an unequivocal division of the nuclear granules is taking place.

Spore formation proceeds in the following manner. The nuclear granules, which in the vegetative stages are scattered throughout the cell, begin to aggregate at one end. A darkly staining, nucleus-like mass is thus produced (fig. 21*b*). A spore membrane appears round this, and, as it hardens, its staining capacity is gradually lost, so that finally an unstained, highly refringent spore becomes visible at the end of the organism (fig. 21*c*). Only a part of the granules enter into the formation of the spore. The remainder gradually degenerate, and are possibly in part volutine and other non-nuclear substances. Certainly a different intensity of coloration can often be noticed in the granules during even the vegetative stages of existence. Ultimately the whole of the cell-remains without the spore break down, so that the single spore is left with more or less of the dead cell attached (fig. 21*d*). Germination from the spore I have not observed.

Spore formation has been described previously in several Spirilla, e.g. in Sp. sporiferum, Mig., and in Sp. endoparagogicum, Sorokin. The details of the process are not known, however. Babès also found a Spirillum which sporulates, and he has figured it with deeply-stained masses at the ends during this process—resembling somewhat the

single terminal mass of nuclear granules in the spore formation of Sp. monospora.

Spirillum monospora remains actively motile until the spore is fully formed. It then presents a very characteristic appearance, as it swims about bearing its glistening, terminal spore.

IV. Spirochæta bufonis, n.sp. (Pl. 6, figs. 22*a*, *b*.)

I have little to say regarding this organism beyond recording its presence. It is very rare, so far as my experience goes. I have found it but once, in the rectum of Bufo vulgaris, L., and then in only very small numbers. It measures about 8—10 μ in length by about 1·5 μ in breadth. There are very few turns to the spiral, which displays some irregularities owing to the flexibility of the organism. The average distance between the turns is about 3 μ.

An undulating membrane or periplastic sheath can usually be seen extending from one end to the other. The spirochæt resembles S. buccalis, Cohn., but I have never seen terminal flagella, which are described in this species. The organism is very actively motile.

No internal structure has been satisfactorily demonstrated. A granular appearance of the protoplasm is usually to be seen, somewhat resembling that figured by Jaffé in S. culicis —another gut-inhabiting spirochæt, from Culex larvæ.

V. Treponema (?), sp. (Pl. 6, fig. 23*a*, *b*, *c*, *d*.)

An organism which appears to belong to the genus Treponema, Schaud., has been found by me on two occasions in the large intestine of the toad, B. vulgaris, L. Both animals were captured in the same locality, and contained the parasite in large numbers.

This organism is more difficult to observe in the living state than any other which I have ever encountered. It is

very active, and but feebly refractive. Its length varies from ca. 5—12 μ, whilst its breadth is exceedingly hard to gauge. From measurements of stained preparations, I judge it to be approximately 0·2 μ. The protist is flexible and spirally twisted, the distance between the turns of the spiral being about 2 μ. Some thicker forms are occasionally to be seen (fig. 23*a*), and others of an extraordinary slenderness (fig. 23*b*). In others (23*c*) the ends appear to taper into an exceedingly delicate filament, possibly a flagellum.

I have stained this organism with Heidenhain's iron-hæmatoxylin, and also with Giemsa. With the latter it stains a pale pink.

This protist bears a remarkable likeness to Treponema pallidum, Schaud., first described by Schaudinn, and since observed by many other workers, in syphilitic lesions. Whether these two forms are really related or not can only be decided by a knowledge of their life-histories. And unfortunately, despite the work of Krzysztalowicz and Siedlecki, and of many others, there is but little of the life-history of Schaudinn's organism known with certainty. I think it therefore premature to name this parasite from the toad.

Of its life-history I know nothing. Some of the longer forms show a thin place towards the middle, which might be due to division—either transverse or possibly the end of longitudinal. Even in the spirochæts—e. g. in S. balbianii—the method of division is disputed. For, although Perrin is assured that it is longitudinal, Swellengrebel believes it to be transverse, and regards longitudinal division as unproved. The appearances figured in T. pallidum by Schaudinn and many others certainly suggest that in this organism at least a longitudinal splitting is the rule.

I have purposely avoided discussing whether the spirochæts, etc., are Protozoa or Bacteria. For it seems to me fruitless to discuss the matter in our present state of ignorance. Indeed, those very characters which are taken by Schaudinn, Hoffmann and Prowazek and others to indicate their Protozoan affinities, are said by Swellengrebel to show

their relation with the Bacteria. I prefer, therefore, to employ the non-committal name of "protist."

Appendix.

On the Methods Employed in the Examination of Bacteria and Allied Organisms.

The methods usually employed by the bacteriologist for purposes of medical diagnosis are far too crude for the cytologist. It is not likely that a dried, plasmolysed, and flame-fixed mummy of a microbe can furnish much information regarding its structure during life. And, again, too much weight should not be given to observations made upon organisms grown in artificial and abnormal culture media. Such media are of the greatest value in the hands of the breeder of pathogenic Bacteria, but the development of the organisms in them cannot be unreservedly accepted as their normal life-history. The protistologist who would investigate the real ways of life of Bacteria must study them in their native medium, in the living state, and in carefully fixed and stained preparations.

Endoparasitic Bacteria usually survive but a short time in hanging drop preparations. They are much more suitably treated by being rapidly placed on a slide, covered with a coverslip with wax feet, and rapidly waxed round the edge with a small candle. In such preparations, if quickly and carefully made, they will live for a long time. They are best examined by means of a water-immersion apochromatic objective.

Intravitam staining is often very serviceable. The stains which have generally proved most useful are methylene blue, neutral red, and Brillantkresylblau.

All of these stains impart a more or less red colour to the metachromic granules during life.

In order to make good permanent preparations it is often necessary to dilute the medium in which the bacteria live.

Ordinary physiological salt solution usually makes the resulting film too watery to fix in a proper manner, but I have found that salt solution containing about 15 per cent. of egg albumen answers admirably. The Bacteria from the large intestine of Amphibia live in this medium for a long time, and very good moist film preparations can be made. Swellengrebel uses a solution of gelatine for the same purpose, and has been successful with Bacteria and yeasts.

The moist films made in this way should be as thin as possible and fixed immediately. For this purpose I know of nothing better than Schaudinn's sublimate-alcohol (2 : 1), used hot. Staining is best accomplished after this treatment by Heidenhain's iron-hæmatoxylin. Very good permanent preparations can be made in this way. I do not find it necessary to wash out the sublimate with alcohol containing iodine, as is usually recommended.

Good results may also be achieved by fixation with osmic vapour or in 1 per cent. osmic acid. And the method of fixation over the vapour from osmic and acetic acids (Plimmer, 'Proc. Roy. Soc.,' B., lxxix, 1907) gives good preparations.

A very good and simple way of fixing is that used by Swellengrebel. A small drop of culture fluid is spread out on a coverslip with a small drop of formalin solution (I use Schering's 40 per cent. formaldehyde) and allowed to dry. I find it best to harden the film in absolute alcohol for about fifteen minutes afterwards. One per cent. osmic acid can be used in the same manner as formalin. After such fixation Heidenhain or Giemsa may be used. Both give excellent results. Giemsa is not so successful after sublimate fixation.

Although drying before fixing is not to be recommended, it sometimes gives quite good results when followed by alcohol fixation and Giemsa—the method used by Perrin for Spirochæta balbianii. Fixation of moist films in absolute alcohol has often proved successful, but it not uncommonly causes plasmolysis.

I have used the following method of staining with Giemsa without drying at any period in the process, and have found

it quite successful:—Expose coverslip (carefully cleaned) to vapour from 1 per cent. osmic acid thirty seconds; spread out small drop of culture on exposed surface, and hold in vapour thirty seconds to one minute; harden in absolute alcohol ten to fifteen minutes; stain in Giemsa (Grübler, 1 drop to every c.c. of water) for twenty minutes; wash in water; dip rapidly into absolute alcohol; take coverslip quickly through three changes of xylol and alcohol (equal parts) into pure xylol; when quite clear mount in cedar oil. I have obtained equally good results with formalin-fixed films treated in this way. And I have also been able to make successful preparations in this manner—without drying—using as stains Löffler's methylene blue and corbol-fuchsin. Dehydration may also be effected without decolorising by using acetone (puriss. acid-free) instead of alcohol (Schridde, C.B. allg. Path. u. Anat., xvi, 1905). I have not been very successful with this method, but I believe with a little modification it could be made very effective.

Giemsa preparations should be mounted in cedar oil or neutral Canada balsam. These preparations are usually the prettiest, but I think iron-hæmatoxylin is more accurate.

Many other methods may, of course, be used in studying Bacteria. I have merely given those which I have myself found useful, in the hope that they may be of some use to others.

Zoological Laboratory,
Cambridge;
5th September, 1907.

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EXPLANATION OF PLATE 6,

Illustrating Mr. C. Clifford Dobell's paper on "Notes on some Parasitic Protists."

[Figs. 1—8 are drawn from life, under the Zeiss 2·5 mm. (apert. 1·25) water immersion objective, compensating ocular 12. The remainder are from permanent preparations stained by Giemsa's method. Figs. 9—19 alcohol fixation, and Figs. 20—23 formalin fixation. Drawn under Zeiss 3 mm. apochromatic oil-immersion (1·40) with compensating ocular 18.]

Figs. 1—19.—Bacillus flexilis.

Fig. 1.—Ordinary vegetative individual, showing finely-granular, somewhat alveolar protoplasm, etc., and in which the outer layer is carefully focused.

Fig. 2.—An individual in which the spiral filament is very clearly seen.

Fig. 3.—Motile organism in which two fully-formed spores are seen.

Figs. 4—7, more enlarged drawings of one end, showing development of spore.

Fig. 4.—Beginning of spore formation.

Fig. 5.—Later stage.

Fig. 6.—Still later, formation of spore membrane.

Fig. 7.—Still later, fully formed spore.

Fig. 8.—A single spore, more highly magnified. A little cap-like projection is seen at one end.

Fig. 9.—Large, flattened specimen, showing chromidia, and faintly striated halo of pink cilia.

Fig. 10.—Division by constriction.

Fig. 11.—Subsequent stage in an organism which is about to sporulate. The constriction is disappearing, and large chromatin granules are arranging themselves in the first stage of a spiral.

Fig. 12.—A similar stage, probably somewhat earlier—less flattened.

Fig. 13.—At this stage the chromatin is all arranged in an irregular spiral.

Fig. 14.—The chromatin is now arranged in two large terminal masses, with an irregular intermediate portion.

Fig. 15.—A later stage, the intermediate part of spiral breaking up.

Fig. 16.—Still later. The terminal masses of chromatin are now definitely rounded off as the spores. They are stained red.

Fig. 17.—The spores are now stained a deep blue, owing to the presence of a newly-formed spore membrane.

Fig. 18.—The spores, as their membrane hardens, stain less deeply. They have moved away from the extremities, which are seen to be stained blue. The intermediate region, however, is now stained pink, owing to the presence of the broken up chromidia.

Fig. 19.—Spores now fully formed; remainder of cell breaking up.

Fig. 20.—Bacillus spirogyra. (*a*), Ordinary individual; (*b*), beginning of division; (*c*), organism which has just divided.

Fig. 21.—Spirillum monospora. (*a*), Normal individual; (*b*), individual in which the granules are collected at one end to form a spore; (*c*), spore now formed, provided with resistant membrane; (*d*), later stage, remains of cell breaking down and leaving the single spore.

Fig. 22.—Spirochæta bufonis. (*a*) and (*b*), two individuals, showing general structure.

Fig. 23.—Treponema-like organism from toad. (*a*), a thick individual; (*b*), a very slender one. In (*c*) a tapering process (? flagellum) is seen at one end.

Studies in Spicule Formation.

VIII.—Some Observations on the Scleroblastic Development of Hexactinellid and other Siliceous Sponge Spicules.

By

W. Woodland,

The Zoological Laboratory, King's College, London.

With Plate 7.

The contents of the present paper, though brief, represent the results of a year's careful inquiry into the manner in which the spicules of siliceous sponges are produced by the silicoblasts of the organism. This subject having already attracted so much attention from spongologists, most of my results necessarily have been to a large extent merely confirmatory of those obtained by previous workers, and these, of course, I shall mention as briefly as possible. A few of my results, on the other hand, are new, and in one or two instances corrective of former work.

My material has consisted of typical examples of both siliceous groups of sponges—the Triaxonida (Hexactinellida) and Tetraxonida (Tetractinellida and Monactinellida). Some of my specimens have been prepared by the osmic acid and picro-carmine method, already described in previous Studies, and others (the majority) with borax-carmine. The former is the better method. The Monactinellida (viz. young portions or buds of Tethya lyncurium, Hymeniacidon sanguinea, Halichondria panicea, Esperella lingua, Siphonochalina coriacea, Axinella polypoides,

Suberites domuncula, Microciona atrasanguinea, Cliona sp. and Ectyon ovoides) and Tetractinellida (viz. young portions of Geodia gigas, Corticium candelabrum, Thenea muricata, Stelletta carbonaria, and Chondrilla nucula) were obtained partly from Naples and partly from Plymouth; the Hexactinellida (viz. young portions or buds of Rossella podagrosa, Kpk., R. antarctica, Ctr., Anoxycalyx ijimai, Kpk., Anaulosoma schulzei, Kpk., Hyalascus hodgsoni, Kpk., and Aulorossella longstaffi, Kpk.) were in greater part supplied to me from the British Museum (the "Discovery" material) at the suggestion of R. Kirkpatrick, Esq.,[1] and by permission of Professor Sir E. Ray Lankester, and the rest (viz. young portions of Euplectella marshalli, Acanthascus cactus, and Crateromorpha meyeri) were sent to me at my request by Professor Ijima from Tokyo. I wish to thank Sir E. Ray Lankester, Mr. R. Kirkpatrick, and Professor Ijima for so kindly assisting me in this essential; I also wish to express my indebtedness to Professor Dendy for his ever-ready aid in connection with spicule nomenclature, for references to the literature of the subject, and for kindly reading over the manuscript of this paper.

Hexactinellida.

The scleroblastic formation of hexactinellid spicules has hitherto, so far as I know, been studied solely by Ijima (**15**, **16**) and by Schulze (**31**), and their results have been included in three sumptuous monographs on hexactinellid sponges published in 1901 and 1904. Ijima has described and figured several developmental stages of the hexasters and one or two other types of hexactinellid spicules in Euplectella marshalli and Rhabdocalyptus capillatus; Schulze described and figured the scleroplasmic investment of certain

[1] Mr. Kirkpatrick has recently published a systematic description of these sponges obtained by the National Antarctic Expedition (**1**).

spicules, which are adult, or nearly adult, in Trichasterina borealis.

Notwithstanding the large quantity of material which has been placed at my disposal and my thorough examination of this material, I have been very little more successful than the two investigators just named in discovering the early stages in the development of hexactinellid spicules.[1] The earliest stage which I have found is indeed somewhat younger than that figured by Ijima, but it throws very little, if any, extra light on the question as to the condition of the scleroplasm at the time of origin of the spicule and as to the early morphogenesis of the spicule itself. The young stages which I have figured were present in Rossella podagrosa, Kpk., the histology of which species was comparatively well preserved. I have only figured the scleroplasmic investment of spicules from this and the allied species, R. antarctica, because in the other genera which I have studied the spicules were in precisely the same condition.

Ijima's remarks on the scleroplasm in connection with the young spicules described by him are as follows:—"A hexaster begins its development as a hexactin. . . . The hexradiate principals, during the entire period of development of both the floricome and the graphiocome, are imbedded in a body of protoplasmic substance, enclosing a crowded number of nuclei. This nucleated substance may not improperly be called the scleroblast-mass. . . . At first, so long as the terminals are yet undeveloped or are very short, the mass may be said to present a more or less octahedral shape, with somewhat concave surfaces and with rounded corners. In it the three axes of the principals are disposed similarly to the axes in a crystal octahedron, the outer ends of the principals coming up very close, but I think normally not quite, to the surface at the six rounded corners. The mass may otherwise be described as having its surface raised into six, radially directed, hump-like protuberances by the six prin-

[1] It is worth remarking that the buds of my hexactinellids contained fewer young spicules than the older portions of the sponge body.

cipals contained within. Later, after the terminals have considerably advanced in growth, the scleroblast-mass appears at the centre of the developing hexasters as a more spherical body, not unlike a berry, on account of the aggregated nuclei." "Not a trace of cell-outlines is discernible around them [the aggregated nuclei], which fact makes me believe that the scleroblast-mass represents a syncytium." And, referring to the prevalent but mistaken notion that the scleroblast nuclei change their position in order to superintend the process of deposition in different regions of the spicule, Ijima remarks:—"Certain it seems that, during the growth of the terminals, no nucleus moves away from its group around the spicular centre. At least I could gather no evidence pointing to such a movement. It is true that, after a certain period in the growth of terminals, a variable number of nuclei is met with right away, or in close proximity to, these. However, they are altogether so inconstant in number and indefinite in position that it is exceedingly questionable if they have anything to do with the building up of the terminals." My figures confirm all the preceding remarks. Fig. 1*a* depicts a stage in which the terminals have not nearly reached the surface of the spherical syncytium, and which is, therefore, younger than any figured by Ijima. Ijima goes on to remark concerning the "great probability . . . that each growing terminal is completely invested by an extremely thin protoplasmic layer, specialised physiologically at least as the secretive matrix, and standing in direct continuity with the scleroblast-mass. Such a layer, however, could never be clearly demonstrated."[1] I may say that I have many times observed in my preparations the film of scleroplasm which invests the terminals of both young and old spicules (and which I have endeavoured to represent in figs. 3 and 6), although this is not by any means always visible; in fact, usually is not.

As shown by my figures the scleroblast-mass varies con-

[1] Ijima, however, in a later contribution (**16**) describes and figures this layer in the oxyhexasters of Rhabdocalyptus capillatus.

siderably in size, and this feature is probably related to the different kinds of spicules to be produced. So few really young stages exist in my material that I am quite unable to say of what types of spicule my figures represent the young stages; possibly it would be difficult to say under any circumstances, since the young hexact must be an initial developmental stage common to all types, both megascleres and microscleres.

Further, although I am unable to demonstrate it by actual examples,[1] I may say that I think it is highly probable that the initial granule from which in all probability the six rays of the hexactin grow out, is found within a syncytium, and not, as all other siliceous sponge spicules are, in a single cell. In support of this statement I may adduce the following evidence: (*a*) cell-clusters similar to those represented in fig. 1, are occasionally found in the tissues of the sponge, and are apparently identical with those enveloping young spicules; (*b*) the large number of nuclei present in the syncytium containing the young spicules figured confirms the view that more than one nucleus was present at the origin of the spicule; and (*c*) this distinction between the modes of origin of hexactinellid and other siliceous sponge spicules is only one of many distinctions which separate the Hexactinellida as a group from other siliceous sponges.

The great extension of the rays of the megasclere type of spicule during the later stages of development in every case causes the centrally-situated syncytial mass to decrease in size and in some cases to vanish, since the scleroplasm is needed for peripheral growth. Ijima suggests that, since in calcareous sponge spicules the scleroblasts ultimately desert the spicule, the syncytium of the hexactinellid spicule possibly does the same, and, as is well known, numerous other authors have often asserted that this desertion does actually

[1] I searched with the greatest care for a syncytium containing a siliceous granule, spherical or six-cornered, but, as stated in the text, I met with no well-defined stage younger than that figured.

occur in the case of other siliceous sponge spicules. For several reasons I very much doubt if this assumption of the desertion of the siliceous spicule, hexactinellid or otherwise, by the scleroplasm, is justifiable, at least in the majority of cases. It must be remembered that the scleroplasm which builds up the triradiate and other spicules of calcareous sponges differs from the syncytial scleroplasm of siliceous and, indeed, many calcareous spicules, in that it consists of separate scleroblasts, cylindrical or semi-cylindrical in shape (**5**), which are not united to form a syncytium entirely enveloping the spicule, and which are in consequence free to leave the spicule at almost any period of deposition. On the other hand, seeing that the syncytium which envelops a complex hexactinellid megasclere, e.g. (and the microscleres certainly retain their syncytia) is correspondingly distended and configurated, it is difficult to understand how the syncytium could take its leave as a whole, and it is exceedingly doubtful if the small portions containing nuclear matter separate themselves off from the rest. Personally I believe the scleroplasm of all or, at any rate, most siliceous sponge spicules clings to the spicule as long as it persists, although in many cases it becomes so attenuated as to be invisible. Indeed, the presence of a nucleus is frequently the only visible indication of the existence of a scleroplasmic film enveloping the spicule.

Ijima again suggests the probability that the six rays of the hexactinellid spicule originate as separate sclerites, but supplies no evidence in support of this view, merely mentioning the mode of origin of the triradiates and quadriradiates of Calcarea as possibly presenting an analogous case. That no analogy can be drawn from the mode of development of these calcareous spicules is, I think, sufficiently manifest on remembering that each of the three monaxons of the calcareous triradiate is produced from, and throughout subsequent growth is constantly related to, one of the three cells which "fused" to form the "trefoil" (**4, 5**), a feature entirely absent in the case of the hexactinellid

spicule, in which no "cells" exist, and in which the nuclei of the undoubted syncytium bear no definite relation to the constituent rays. Moreover, as far as I know, there is no authenticated instance of any siliceous spicule arising by the fusion of initially-separate parts contained in an individual syncytium, though of course it may well be that the hexactinellid spicules are peculiar in this respect as in others. The only ground for believing that the hexact spicule arises by the central fusion of six initially-separate rods that I can imagine is the general similarity which many hexactinellid spicules bear to certain Radiolarian skeletons. In Radiolarians these rays are usually separate, at any rate initially, and in some cases they become fused centrally. If the mode of deposition of hexactinellid rays in the spherical syncytium can be shown to be at all similar to that of Radiolarian spines in the spherical body, a reason certainly exists for supposing that the hexact rays may be deposited initially in a separate condition, but there is no other reason for this supposition that I know of, and personally I believe the six rays of the hexact spicule will be found to emerge from a spherical granule.

Tetractinellida and Monactinellida.

Generalising the large number of statements[1] which have been made by the various authors enumerated in the biblio-

[1] To avoid burdening the text with a somewhat unnecessary account of previous work on the scleroblastic development of siliceous sponge spicules—an account of which both Minchin (**4**) and Maas (**24**) have provided an outline—I append a full bibliography of the subject, mentioning after each paper the type or types of spicule, the scleroblastic development of which is described within. I may here add that I have not been able to trace the development of every type of spicule contained in the genera mentioned in the Introduction, and indeed only in one or two cases would this have been desirable (e.g. the development of the rosettes of Esperella), since the development of most types is already known. In fact, so far as I am aware, only in Chondrilla nucula (Tetractinellida), and in Siphonochalina coriacea, Axinella polypoides, Suberites domuncula, Microciona atrasanguinea, Cliona sp., and Ectyon ovoides (Monactinellida), among the sponges

graphy concerning the scleroblastic development of tetraxonid and monaxonid spicules, we may say without hesitation that the vast majority of the spicules, widely as they may differ among themselves with respect to form and size, arise each as a granule contained within a single scleroblast. The few siliceous spicules which do not thus arise from a single centre, but from several granules contained within the scleroblast (e. g. orthodragmata, Carter's bundles of "tricurvates," etc.) are exceptional, and in every one of these cases the "spicule" consists of several parts throughout its existence. I am not aware of a single instance in which the body of a siliceous spicule-individual is built up by the fusion of originally separate parts, i. e. visible parts, though statements have recently been made to the contrary (see below).

Further, with the exception of those spicules which attain a relatively large size (e. g. the monaxons of Esperella, Ectyon, and Tethya, the large tetracts of Geodia and the spherasters of Tethya) all these spicules throughout their existence as skeletal elements of the sponge-body remain enveloped by the distended substance of the single scleroblast in which they arose; in other words, the nucleus of the scleroblast does not divide (figs. 10—18).

It is needless for me to enter into details in connection with the morphogenesis of each type of spicule; it suffices to say that careful work has proved that in the vast majority of cases each spicule, whatever its ultimate shape, originates as a granule, and only by degrees assumes its final form whilst enveloped by the formative scleroplasm. Also, so far as we know, all growth of the spicule is accretionary and never interstitial, and this fact is constantly to be borne in mind when the identification of young forms of certain types of spicule is in question.

As stated above, the building up of siliceous sponge spicule-individuals by the secondary fusion of separate de-

I have studied, has the scleroplasm associated with the spicules not been hitherto described (see figs. 10—17).

posits is unknown; in other words, no one has yet found siliceous equivalents of the triradiates and quadriradiates of Calcarea. This being the case, it was with some interest that I read an account of this very process which was stated to occur in connection with the spicules of Tethya lyncurium. This account was contained in a paper published by Dr. O. Maas in 1900 (**24**). The exceptional nature of the statements in this paper led me to pay special attention to the scleroblastic development of the spicules in Tethya lyncurium, but, as on a former occasion,[1] I was unable to confirm Dr. Maas' results. Maas makes the following state-

[1] To enable the reader to estimate the value of Dr. Maas' reply (**3**) I will recall the facts. Minchin in 1898 (**4**) showed that each triradiate spicule of the Ascon sponges which he investigated originated as three separate rods radiating from a common centre, each ray being produced by a pair of cells of the "sextet," and that these rods only secondarily became fused together at their inner extremities to form the triradiate spicule. In the same year (**1a**) and in 1900 (**2**) Maas published papers, each dealing in part with the similar spicules in Sycons. In the latter paper (and with this I am chiefly concerned) Maas described a process of spicule formation as occurring in Sycons which is radically different from that which occurs in Ascons—each triradiate spicule, as a whole, being stated to arise as a single concretion in one mother-cell. At Professor Minchin's suggestion I, during 1903 and 1904, devoted the greater part of a year's work to ascertaining for certain whether the process of spicule formation in Sycons was so fundamentally different from that obtaining in Ascons, as alleged by Maas, and I found, both to Professor Minchin's and my own satisfaction, that the mode of spicule formation in three species of Sycon closely allied to those studied by Maas was identical with that found in Ascons. This being the case I considered that Maas' statements were erroneous, and I ventured to express that opinion—perhaps in too brusque a manner. Dr. Maas in his counter-criticisms says that I obtained anomalous results owing to the method of fixing the Sycons which I adopted—a criticism which applies to Professor Minchin's work as well as my own. I can only reply that Professor Minchin's slides (and my own to a less extent) have been inspected by numerous zoologists, and that no fault has ever been found with them on the score of imperfect histological preservation. Maas' criticism simply proves, what indeed he admits, that he has never seen the three-rod stage of the triradiate spicule, else he would not assume it to be an artefact. Maas again speaks of the initial stages of spicule deposition as if, owing, as he states, to their small size and transitoriness, it were almost impossible to observe them. This is not the case. They are easily to be observed, pro-

ments: (*a*) the tylostyle spicule arises by the fusion of separate granules present in the substance of the mother-scleroblast; (*b*) the chiaster spicule arises by the junction at their inner extremities of several rods of silica which are deposited in the scleroblast so as to lie radially in the cell-sphere; and (*c*) the spheraster usually arises by the fusion of two or more calthrops (each of which arose as a granule contained by a single scleroblast), which serve as a basis on which the rest of the silica is deposited—from which it is inferred that Tethya has tetractinellid affinities. This last statement is illustrated by a somewhat extraordinary figure (his fig. 33) of two closely-apposed attenuated calthrops spicules, each contained in its scleroblast. It is significant that if these three statements be true, then, as before stated, spicule formation in Tethya lyncurium proceeds in a manner different from that hitherto found in any other monactinellid, or, for that matter, tetraxonid sponge.

With regard to the first statement, I entirely agree with Weltner's estimate of its value (**38**). Weltner rightly considers that the several granules have nothing to do with the tylostyle spicule, and that this arises, like other siliceous monaxons, from a single granule which gradually elongates and assumes the form of the adult spicule by continued deposition of silica on its surface. Further Maas' figures of

vided that plenty of suitable material, i. e. a quantity of very young Sycons, is to hand, that this material is prepared in the way described by Minchin, and that the observer possesses sufficient patience to find the comparatively few young stages in his preparations. Maas' remarks about what he supposes to be my ignorance of his own paper and other relevant literature are incomprehensible to me since, besides being entirely mistaken, they are entirely irrelevant to the subject under discussion. Because, as Maas points out, I adopted Minchin's convenient summary ('Zoological Record' for 1901) of his statements concerning spicule formation (and Maas does not dispute the accuracy of Minchin's rendering of his statements) I fail to see that anyone is justified in drawing the curious conclusion that I was unacquainted with the paper which I was criticising! I may add that in my previous paper (**5**) I inadvertently ascribed "tuning-fork" spicules to Clathrina lacunosa.

the cells in connection with the adult tylostyle are quite incorrect. These never have either an epithelial[1] disposition or the "syncytial" disposition shown; on the contrary, each of the several "cells" (i. e. nucleated portions of the syncytium) in connection with the elongated spicule forms a sloping mound-like mass containing the nucleus, and lies in close apposition with the spicule, as depicted in my figure of the Esperella monaxon (fig. 19).

Maas' second statement I believe to be not more valid than the first. The chiaster, according to my observations, originates, like the spheraster, as a granule contained within the cell, and this granule, also like that of the spheraster, develops rays on its surface (fewer in number[2] and relatively longer and more uniform in thickness as compared with those of the spheraster) which become those of the adult chiaster (figs. 20 *c*, *g*). The mother-cell, as Maas states, remains undivided.

Maas' third statement is also contradicted by my observations. I can find no evidence whatever that the spheraster ever passes through a calthrops stage; on the contrary, it develops in almost exactly the same way as the chiaster. The initial granule develops numerous radiating rays whilst quite small (Maas' fig. 7 is quite correct e. g.), and from this ground form (figs. 20 *b*, *d*) the adult spicule is formed (figs. 20 *h*, *j*, *k*) by the further accretion of silica, and also, I believe, by the occasional development of fresh rays during the early stages. As implied above, these rays of the spheraster are more numerous and more conical in form than those of the chiaster, and these differences alone distinguish the younger developmental stages; at first indeed it is very

[1] Maas makes the same mistake in connection with the monaxon spicule of Sycandra, figuring the half dozen scleroblasts as spherical cells just touching the surface of the spicule. Scleroblasts never have this arrangement in connection with spicules or, so far as I know, elsewhere.

[2] Even if, as occasionally may be the case, these spines of the developing chiaster are four in number, this fact affords no justification for Maas' statements in connection with the spheraster.

difficult to distinguish between them. The development of the Tethya spheraster in fact is essentially the same as that described by Keller[1] (**18**) for the spheraster of Chondrilla nucula. I may further mention that Miss Sollas (**36**) also failed to find any signs of calthrops, though plenty of globules, in the larva of Tethya ingalli. The single scleroblast which contains the initial granule divides as the spheraster increases in size, with the result that the adult spheraster possesses several cells; whether any of these are not division-products of the original mother-cell but are extraneous in origin, as Maas confidently thinks, I am unable to say, and I fail to see how it is possible to say without at least very special inquiry.

With the above criticism on Maas' work in connection with spicule formation I may include another and minor one. During my observations of the developing spicules of Esperella lingua I could never find more than one nucleus associated with each of the anisochelæ in this species (fig. 18) whereas Maas (**23**) states that four nuclei occur in connection with each such spicule in Esperella lorenzi—a statement which I feel sure is incorrect. I have observed numerous rosettes in E. lingua, and in every case each anisochele only possessed one nucleus. In each rosette the anisochelæ were embedded at their inner extremities in a spherical mass of protoplasm which contained several nuclei, but all of these nuclei were quite distinct from those which belonged to the anisochelæ. The answer to the question as to how the anisochele rosette is formed is not as yet known. Carter (**8**), with others, supposes that the entire cluster of anisochelæ is derived from separate silica deposits initially present in one

[1] Or rather as Keller should have described it. Keller figures the spikes as arising successively on the surface of the siliceous globule, whereas, in reality, they arise for the most part simultaneously as small knobs which gradually assume the pointed form. The spicule is enveloped in one scleroblast throughout its existence. The calthrops of Corticium candelabrum e. g. also originates in the same way—the granule developing four processes which elongate to form the four rays of the calthrops; also the chiaster, oxyaster, and sterraster of Geodia gigas.

cell, and even states that he "observed in one cell two inequianchorates together end to end or slightly overlapping each other; but this was in the equianchorate stage." Whether this interpretation of the origin of the rosette is or is not the correct one future inquiry must determine. It is quite certain that the trichites of the ortho- or trichodragmata are produced in one cell and remain in one cell throughout their existence, i. e. the nucleus never divides. Carter also describes and figures the production of several "tricurvates" (toxas) in one cell, the nucleus of which similarly never divides, and several other kinds of dragmata are known. I think it highly probable that subsequent inquiry will prove the Esperia rosette to be derived from a cell-cluster, each of the peripheral cells of which produces an anisochele, the central cells remaining as the central multinucleated "spherical mass of protoplasm" described above.

In conclusion I may remark upon the significant fact that although all sponge spicules arise in the interior of cells, yet in each of the three great groups of sponges—the Tetraxonida, Triaxonida, and Calcarea—spicule formation proceeds on very different lines. In the Tetraxonida the spicule typically arises and continues to exist in one cell, i. e. the cell entirely envelops the spicule; in some cases the nucleus of this cell divides so that a syncytium is formed, and in a few instances several "spicules" are produced in the interior of a single cell. In Triaxonida all the evidence points to the conclusion that a large spherical syncytium containing many nuclei must be formed before the spicule is secreted in its interior, and that the three-dimensioned spicule (the six rays of which arise as outgrowths from an initial granule) is situated in this syncytium, at least for some period of its growth, in much the same way that certain Radiolarian spines are enclosed in the spherical Radiolarian body. Finally in Calcarea, at least the majority of spicules (i. e. with the possible exception of certain monaxons) are formed by the apposition of cells in twos (either by cell-division or cell-junction) and threes (solely by the junction of separate cells) which do not entirely envelop

the spicule as in the other two groups, but merely adhere to the spicule-ray as cylinders or semi-cylinders. These differences as regards the method of spicule formation in the three groups of sponges doubtless possess phylogenetic significance.

Summary.

1. The earliest stages in development of the Hexactinellid spicule are at present unknown, but there exist reasons for supposing that it originates as a granule enclosed by a spherical syncytium in which cell-outlines are absent, and that the six rays of the hexact grow out from this granule.

2. The earliest stage of development yet discovered is the small hexact, the rays of which do not extend to the periphery of the enveloping syncytium.

3. The rays of the hexact elongate, causing the spherical syncytium at first "to present a more or less octahedral shape, with somewhat concave surfaces and with rounded corners" (Ijima).

4. The rays at length extend beyond the spherical contour of the syncytium (the scleroblast mass), the scleroplasm of which, however, persistently adheres to the rays as a thin film, which occasionally includes nuclei.

5. The peripheral growth of the megasclere type of spicule causes the spherical syncytium enveloping the point of junction of the six rays to dwindle and finally to disappear on account of the distension involved. The whole of the microsclere, with the exception of the terminals, remains permanently enveloped by the spherical syncytium.

6. All tetractinellid and monactinellid spicules originate as granules contained within single cells. In a few instances the spicule arises from several granules within the cell and then consists of separate parts (dragmata, for example).

7. All growth is accretionary. There is no well-authenticated instance of a siliceous sponge-spicule being formed by

the fusion of at-first-separate parts—such as occurs in calcareous sponges, for example.

8. With the exception of very large spicules, the nucleus of the distended scleroblast remains single throughout growth.

9. It is a significant fact that spicule formation proceeds on very different lines in each of the three great groups of sponges—the Tetraxonida, Triaxonida, and Calcarea.

Note.—Unless exceptional opportunities for further work in connection with spicule formation should present themselves that it is intended the present Study shall conclude the series.

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EXPLANATION OF PLATE 7,

Illustrating Mr. W. Woodland's "Studies in Spicule Formation." (VIII.)

HEXACTINELLIDA.

Figs. 3, 5, and 9 are from preparations of Rossella antarctica, Ctr.; the remainder (figs. 1, 2, 4, 6, 7, 8) are from R. podagrosa, Kpk.

Fig. 1 (× 1600 diam.).—Young hexact stages of development. 1 *a* represents a hexact small in comparison with the enveloping syncytium. The pink hue of the spherical mass of scleroplasm is due to the presence of numerous nuclei which, owing to imperfect preservation of the tissues, are not always visible individually.

Fig. 2 (× 1600 diam.).—Microscleric monodiscohexaster with somewhat dilated extremities. It is not certain whether the extremities have yet assumed their final form, but it is probable.

Fig. 3 (× 2600 diam.).—Adult microscleric holoxyhexaster. Two nuclei have migrated from the central spherical mass of scleroplasm.

Fig. 4 (× 1600 diam.).—Holoxyhexaster, about half-grown.

Fig. 5 (× 1250 diam.).—Adult microdiscohexaster, with several peripherally-situated nuclei ("cells"). Each terminal is invested with a thin film of sclèroplasm, though this is not always visible.

Fig. 6 (× 2000 diam.).—Half-grown and adult forms of another variety of microdiscohexaster.

Fig. 7 (× 1600 diam.).—Central portion of an oxydiactine, showing the enveloping scleroplasm with distributed nuclei. The position of the four aborted rays is indicated by the mid-way swellings and axial cross.

Figs. 8 (× 700 diam.), 9 (× 625 diam.).—Hexactines with the enveloping scleroplasm well shown.

MONACTINELLIDA.

Fig. 10 (× 800 diam.).—Tylostyle of Microciona atrasanguinea in its scleroblast.

Fig. 11 (× 1600 diam.).—Acanthotylostyle of M. atrasanguinea in its scleroblast.

Fig. 12 (× 800 diam.).—Tylostyle of Suberites domuncula in its scleroblast.

Fig. 13 (× 1600 diam.).—Oxeote of Siphonochalina coriacea in its scleroblast.

Figs. 14, 15 (× 1600 diam.).—Stylus and oxeote of Axinella polypoides in their scleroblasts.

Figs. 16, 17 (× 1600 diam.).—Acanthoxeote and spiraster of Cliona sp. in their scleroblasts.

Fig. 18 (× 800 diam.).—Anisochela (one of a rosette) of Esperella lingua in its scleroblast.

Fig. 19 (× 800 diam.).—Portion of tylostyle of E. lingua showing two of the several nuclei present in the syncytium enveloping the spicule.

Fig. 20.—Development of the chiaster and spheraster of Tethya lyncurium. *a* and *e* represent the first appearance of the spicule as a siliceous globule. The rays appear on the surface of these globules much earlier in some cases than in others; cf. e. g. figs. *b* and *e*. As stated in the text, it is impossible to distinguish between the very young stages of the chiaster and the spheraster. A comparison of fig. *g* with figs. *h* and *j* shows well, on the other hand, that it is quite easy to distinguish adult chiasters from young spherasters of the same size.

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BEYER PROFESSOR OF ZOOLOGY IN THE UNIVERSITY OF MANCHESTER;

AND

E. A. MINCHIN, M.A.,

PROFESSOR OF PROTOZOOLOGY IN THE UNIVERSITY OF LONDON.

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Investigations on the Development of Trypanosomes in Tsetse-Flies and other Diptera.

By

E. A. Minchin,

Professor of Protozoology in the University of London.

With Plates 8—13 and two Text-figures.

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I. Introductory.

In this memoir I propose to give a full account of the work done and the results obtained by me during my stay in Entebbe, Uganda, as a member of the Sleeping Sickness Commission from the beginning of April to the beginning of

December, 1905. Some of my results have already appeared in the form of preliminary reports; but with regard to some few points further study necessitates revision or correction of statements previously made. In my eight months in Uganda I accumulated a great deal of material which it has required much time and labour to work out. The study of this material has occupied the time left from other duties since my return from Uganda, and may now be considered complete.

The commission with which I went to Uganda was to study the life-cycle of Trypanosoma gambiense in its relation to the local species of tsetse-fly, Glossina palpalis. In view of the well-known life-cycle of the malarial parasite, as well as of the remarkable development described by Schaudinn (**40**), for the trypanosomes of the little owl (Athene noctua), it seemed probable that a study of the sleeping sickness trypanosome in its passage through the tsetse-fly would reveal an interesting developmental cycle. Unfortunately, these expectations have not been fulfilled, and, so far as the development of Trypanosoma gambiense is concerned, my investigations have been barren of results, and have yielded conclusions for the most part of a negative character. Incidentally, however, I have made some observations which are, perhaps, not without interest, on another species of trypanosome occurring in the fly, and a record of my work and its results may be of some use if only as a guide to future investigators in this field to enable them to avoid my failure.

In the exposition of the numerous and complicated data furnished by an investigation of this kind, it is difficult to steer clear between, on the one hand, too much subjective interpretation, which may become misleading; and, on the other hand, excessive elaboration of detail, which becomes tedious and difficult to follow. I propose, therefore, to divide this memoir into two chief parts: I shall first set forth the development, so far as I have observed it, of the two species of trypanosomes in a connected manner, and shall discuss the general question of the transmission of trypano-

some infections; after which I shall give a bare record, in chronological sequence, of my experiments and observations. The latter part is intended rather for reference, and to avoid the possible danger of omitting something which in the present state of our knowledge seems immaterial, but which may prove in the future of greater importance.

Before proceeding to describe my results it may be permitted to me to enter upon a brief personal explanation of my relation to the work of the Commission; after which I shall say a few words upon the methods of investigation employed.

When I came to Entebbe, at the beginning of April, 1905, my two colleagues, Lieutenant A. C. H. Gray, R.A.M.C., and the late Lieutenant F. M. G. Tulloch, R.A.M.C., were already at work upon the subject which I was sent out to investigate, and had discovered some facts of importance. In particular, they had found and studied the vast swarms of trypanosomes which are frequently found in the alimentary canal of freshly-caught tsetse-flies. Neither my colleagues nor myself had at that time any doubt but that these "wild" trypanosomes represented stages of T. gambiense. Since it was arranged that Gray and Tulloch were to work in connection with me, and since I did not wish to absorb, as it were, into our joint work anything of which the credit belonged to them independently, I requested them to write up and publish all that they had found before my arrival, so that we could start our collaboration on a clear footing. This they did, and the result was the memoir (17) published in the 'Sleeping Sickness Reports,' a memoir in which Tulloch's excellent drawings were done scanty justice in the reproduction. While Gray and Tulloch were engaged upon this report I commenced a systematic investigation of the anatomy of Glossina palpalis, then an untrodden field of study, upon which a preliminary report was published in the 'Proceedings of the Royal Society' (27). One of the first results of my dissection of the fly was to show that the structure termed by Gray and Tulloch in their memoir "the salivary gland" was really the

proventriculus, although I myself fell into the error of calling it the stomach in my communication.

Our further studies were divided between us as follows: Gray and Tulloch continued the investigation of the "wild" trypanosomes. I confined myself to a study of the changes undergone by T. gambiense when taken up by the tsetse-fly from infected animals. My intention was to work the development of T. gambiense from the beginning, so to speak, up to the condition found by Gray and Tulloch in the fresh-caught flies, and so link my researches on to theirs, with hope of thus getting the complete cycle. This expectation was not fulfilled, since more extended knowledge forced the conclusion slowly, but irresistibly, upon us, that the "wild" trypanosomes had nothing whatever to do with T. gambiense, but represented at least two distinct species occurring in the fly quite independently of sleeping sickness. As a result, however, of our subdivision of the work, my two colleagues accumulated an abundant material of the "wild" trypanosomes; my material of these interesting forms consists only of those found incidentally in flies used for experiments with T. gambiense, in all six flies having been found by me thus infected.

I returned to England at the end of 1905, leaving Gray and Tulloch still at work upon these problems. Our ever increasing doubts as to the true nature of the wild trypanosomes led to our planning a number of crucial experiments, which were carried out by Gray and Tulloch on the island of Kimmi after my departure. The result was to demonstrate conclusively the distinctness of the "wild" trypanosomes from T. gambiense. Meanwhile I had written, shortly after my return to England, an interim report on my work at Entebbe, for the information of the Tropical Diseases Committee of the Royal Society. This report was also utilised by me in my candidature for the chair of Protozoology, which I now hold, and a printed copy of it was sent by me to my friend M. Mesnil, of the Pasteur Institute, who, not being aware of its private nature, of which he had not been

informed, inserted an abstract of it in the 'Bull. Inst. Pasteur.' I am indebted to M. Mesnil's happy indiscretion for securing to me the priority of the statement, that the "wild" trypanosomes of Glossina palpalis were quite distinct from those of sleeping sickness—a conclusion to which Novy came independently after studying preparations sent him by Gray; his results were published in a memoir (**32**), in which he named one of the wild species T. grayi.

Shortly after this came the appalling news that our colleague, Tulloch, had become infected with trypanosomes. This most sad event, of course, put an end to further work, and Gray returned to England, bringing Tulloch with him. We then wrote a preliminary account of our results (**29**), which, so far as poor Tulloch was concerned, was a posthumous work when published. Our collaboration then broke up, and I began the detailed study of my material, and discovered the encystment of T. grayi, a result which I at once communicated to the Royal Society (**28**). It is a common human weakness to attribute failures to bad luck; nevertheless I cannot but deeply regret that I did not discover the encystment while I was in Entebbe. Had I done so, it would have modified the whole course of my researches. The fly in which this discovery was made was one of the last examined by me, a fortnight before I left off working. As I was then professor at University College, however, it was imperative upon me to return to my duties, having already had my leave of absence generously extended by the College for an extra three months.

Such is, in brief, the history of the conditions under which these investigations were carried on. It only remains for me to perform the pleasant duty of thanking those who have helped in my task. To the Tropical Diseases Committee of the Royal Society, in the first place, I am indebted not only for sending me to Uganda, but for providing me with assistance in working through my material after my return. Without the help and experienced advice of my colleagues on the Commission, Gray and Tulloch, I could have done little.

Since my return to England, my two assistants, Dr. J. D. Thomson and Dr. H. M. Woodcock, have rendered me much service in various ways, and Miss E. Y. Thomson has been of great assistance in the tedious task of searching through blood-films for trypanosomes and making counts of them. To each and all of these I desire to express my obligations and return my best thanks.

Methods of investigation.—I commenced my work, as I have said, by making a study of anatomy of Glossina palpalis. In undertaking this I was influenced largely by Schaudinn's work (**40**) on Trypanosoma noctuæ, in which he describes extensive migrations of the trypanosomes in the body of the invertebrate host. In tsetse-flies, however, I never found trypanosomes outside the alimentary canal, in spite of much searching; in this point my results agree with those of Stuhlmann (**41**).

My report on the anatomy of the fly (**27**) contained some errors which I desire to correct; they have been corrected in the reprint in the Reports of the Sleeping Sickness Commission. All through I used the term stomach for what should have been called the proventriculus; in this memoir I shall use the latter term. The true stomach in Glossina is represented by the first part of the digestive tract in the abdomen, that is to say, by the coils which in my figures and description were numbered 5, 6, 7. It is this region which becomes congested with blood after feeding, and in which the blood retains its bright red colour. From the stomach the blood passes into the next coils of the digestive tract, which constitute the true intestine, and here it becomes blackish in colour. Hence we may conveniently speak of the red blood, meaning that in the stomach, and the black blood, meaning that in the intestine proper. The red blood is thick and jelly-like, very difficult to smear out unless broken up with salt-citrate solution[1]; the black blood, on the contrary, is very fluid and watery, the number of corpuscles is more or

[1] Made up as recommended by Laveran and Mesnil (**23**); ·5 gr. sodium chlorine + ·5 gr. sodium citrate + 100 cc. H_2O.

less diminished, and there are usually numerous square crystals in it. The black blood stops short at the point where the Malpighian tubules arise, and where the proctodæum commences. The proctodæum contains no blood, but a yellowish fluid in which are suspended numerous coarse granules showing Brownian movement. Hence in a fly about twenty-four hours after feeding the three regions of the abdominal gut, namely, stomach, intestine, and proctodæum, are marked out by their respective colour—red, black, and pale yellow—in a way to make them easily discernible with the naked eye.

In the examination of a tsetse-fly for trypanosomes I usually began by inserting a fine capillary tube into the pericardial space, in order to draw up some of the fluid circulating in the hæmocœle. It was usually possible to draw up a small drop of the fluid. Some flies, however, were very anæmic and dry; such flies always proved on further examination to be sickly, and usually contained great numbers of bacteria in the gut, in which no blood as a rule was found. The hæmocœle fluid contained always peculiar amœboid corpuscles, generally more or less fusiform, with each end prolonged into a pseudopodium-like process; sometimes one end would show two such processes. The corpuscles showed distinct changes of form. No trypanosomes, however, were found in the hæmocœle. The next step would be the removal of the dorsal integument of the abdomen, and the examination of the contents of the salivary glands and the Malpighian tubules. The salivary glands contain numerous corpuscles or spherules of circular contour, varying from about 9 to 15μ in diameter, imbedded in a granular matrix. Each corpuscle contains an irregular spot towards the centre, usually angular in shape and looking like a split or space. With picrocarmine the corpuscle takes a pink tinge and the central spot a deeper colour. In the fresh condition the salivary corpuscles are about the same size as the fat-globules liberated from the fat-body, but distinguishable from them by their less refringent appearance and also

by the fact that the fat-globules float upwards, while the salivary corpuscles lie at the lowest focus. The Malpighian tubules contain coarse, yellowish-brown granules similar to those found in the proctodæum. Neither in the salivary glands nor in the Malpighian tubules did I ever find trypanosomes or any bodies other than those already mentioned. As I have already stated, the statement of Gray and Tulloch (**17**), to the effect that they found trypanosomes in the salivary gland must be corrected; I was present when they made the observation, and saw the preparation; by salivary gland was meant proventriculus.

After the Malpighian tubules I next examined the genitalia, either testes and seminal vesicles, or ovaries, receptacula, and oviducts (including uterus and embryo, if present). Here also I found in no case anything but the proper contents of these organs, which I need not describe in detail.

The last process is to dissect out the gut for its whole length and to divide it into its four regions, namely (1) the proventriculus with the thoracic intestine and sucking stomach, (2) the stomach with the red blood, (3) the intestine with the black blood, and (4) the proctodæum and rectum. In none of the flies dissected by me did I find trypanosomes in the proventriculus, but Gray and Tulloch, with their more abundant material of T. grayi and T. tullochii, frequently found these species in this part. No trypanosomes were ever found in the sucking stomach,[1] which in the normal condition is filled with air, but which immediately after feeding is found to contain traces of blood, and is sometimes quite full of blood, though this is a rare condition, and perhaps the result of some functional derangement of the organ. The fly, when feeding, appears to fill the sucking stomach with blood, and then to expel it thence into the digestive tract. It can be observed that when sucking blood the fly at intervals raises its head up a little, thus

[1] Gray and Greig (**18**) report trypanosomes in the "ventral food reservoir" (sucking stomach?) twelve hours after feeding. Stuhlmann (**41**) prefers the name "crop" for the sucking stomach.

partially withdrawing the proboscis from the skin of its victim, and then lowers it again.[1] Probably when the proboscis is lowered the sucking stomach is being filled, and when it is raised the blood is being expelled from the sucking stomach. This would account for the traces of blood found in the sucking stomach, and also in the proventriculus and thoracic intestine, after a meal. If the fly over-fed itself, it might not be able to discharge the last bolus of blood from the sucking stomach.

When the digestive tract is gorged with blood it is so distended, and the wall is so thin, that it is a difficult matter to dissect it out without rupturing it and letting blood escape. My colleagues, Gray and Tulloch, taught me a simple and effective method for doing this operation, which is as follows: The terminal segment of the abdomen is snipped off, the body is laid flat on an ordinary slide, and then a mounted needle, seeker, or other suitable instrument, is pressed down flat on the waist or base of the abdomen and passed along with an even, steady pressure towards the tip of the abdomen, so as to squeeze all the contents of the abdomen out on to the slide. With a little care and practice all the abdominal organs can be squeezed out quite uninjured, and can be separated from one another on the slide afterwards. In most cases the thoracic intestine and proventriculus are pulled out together with the abdominal contents. This simple method is most useful for rapid examination of a number of flies.

In order to make smears of the stomach-blood, it is necessary, as already stated, to mash it up with a little salt-citrate solution; for the intestinal and proctodæal contents this is not necessary. It is best to avoid, as much as possible, the use of salt-solutions. By comparing preparations made from pure blood with those of blood that had been mixed with citrate solution, I found the trypanosomes distinctly altered in form in the latter. Our method was to draw up the intestinal

[1] "Der Rüssel wird . . . während des Saugens häufig sägend auf- und abbewegt," Stuhlmann (**41**, p. 4). My statements in the text were written before Stuhlmann's memoir came to hand.

contents into capillary glass tubes, from which fine drops were expelled by blowing them gently on to slides, in order to make the smears. When made, the smears were sometimes dried rapidly in the air and then fixed with absolute alcohol or methyl alcohol, sometimes fixed with osmic vapour, employed in the manner recommended to me by my friend Dr. Plimmer: twenty drops of 4 per cent. osmic acid, with one drop of glacial acetic, placed in a stoppered tube of sufficient calibre to hold a slide, and the wet smear placed into the tube for about half a minute. I also tried a modification of this, as follows: A drop of blood on a slide was exposed, as rapidly as possible, to the osmic-acetic vapour for about half a minute, then mixed with an equal-sized drop of equal parts of fresh serum and dilute glycerine, and the whole smeared out. Subsequently the smear was fixed with absolute alcohol, without letting it dry; the glycerine was used in order to keep it moist for any length of time. I found, however, that this method tended to shrink the trypanosomes, though successful in other ways. It would be necessary to experiment in order to find the exact proportion of glycerine that should be mixed with the serum. In general I found the form of the body much more perfectly preserved in osmic-fixed smears than in air-dried preparations; the trypanosomes appear solid and plastic in the former, and always more or less flattened out in the latter. It seems, indeed, inevitable that the violent method of drying must deform a soft protoplasmic body.[1]

I wasted, I am sorry to say, a great deal of precious time trying to stain trypanosomes by the ordinary technique, which gives such good results with other Protozoa, especially the various carmine and logwood stains, which all proved useless. It is a puzzle to me why the Protozoa parasitic in blood should be so entirely different from other Protozoa in their staining reactions. I have always experienced just the same difficulty in trying to stain other Protozoa by the methods so successful for blood parasites. I fell back, finally, entirely upon the

[1] Lühe (**26**, p. 70), makes some valuable remarks on this point. Compare also Plimmer (**35**).

classic Romanowsky stain, using either Leishman's method, Laveran's Bleu-Borrel method, or Giemsa's stain. Latterly I used Giemsa's stain entirely, differentiating with tannin-orange solution (Unna's, obtained from Grübler). As a rule the smears were "refreshed" with fresh blood-serum, after the procedure recommended by Leishman. I kept my smears uncovered. Those that have been much used for study are now, I find, deteriorating, but those that have not been much looked at seem to be quite unaltered. Frequent baths of cedarwood oil and xylol alternately effect the stain after a time. Some smears, owing to pressure of time, were left unfixed, and were fixed after my return; none of these were very good.

Parasites of Glossina palpalis.—I examined the contents of the digestive tract and other organs in freshly-caught hungry flies with the object of making myself acquainted with the native parasites, if any, of the fly, in order to avoid confusing with them the stages of trypanosomes. Except for bacteria, however, the fly was very free from internal parasites. No gregarines or other sporozoa were found; it would, indeed, be improbable that a tsetse-fly, which apparently feeds exclusively on the blood of vertebrates, should acquire an infection of such parasites, which, as a general rule, are taken up with food in an encysted form by their hosts.

The commonest object in the gut was a large bacillus (figs. 111–123), apparently always present, sometimes in enormous numbers, especially in the stomach. When plentiful they occurred in masses or bundles; in such cases the flies always appeared sickly, emaciated, and anæmic, and usually had the digestive tract empty of blood, even when they had been put on to an animal; whether through the fly refusing to feed, or through rapid absorption of the blood by the bacteria, was doubtful. These bacteria show, apparently, only Brownian movement.[1]

[1] Stuhlmann (**41**, pp. 38, 39), has seen these or similar bodies in G. fusca. He believes them to be protozoa and not bacteria, and considers that they are not parasites because they are found to be invariably present. I cannot agree with him in either of these conclusions.

In the proctodæum and hinder part of the intestine I noted "sausage-shaped protoplasmic bodies, apparently flagellate, swimming actively" (figs. 124, 125). These were perhaps a form of the bacterium already described, as I noted others that appeared to be intermediate between the two forms.

In the same regions I noted also in one fly slender thread-like organisms, wriggling actively; perhaps spirochætes. I never found them, however, in my smears. In one fly (batch of Oct. 1st, 1905, examined Oct. 5th) I noted the red blood in the stomach "swarming with small motile bacteria, not the large inert forms ordinarily found." This fly was one bred out in the laboratory.

I frequently found, in the blood taken from tsetse-flies, and in my smears, curious bodies apparently of vegetable nature; figs. 127–131 show rough sketches from life of these bodies; fig. 126 is from a smear, drawn with the camera lucida, magnified 2000 linear. As may be seen, they are more or less spindle-shaped bodies, sometimes enclosed by a distinct membrane, sometimes not; the contents are divided into two, four, or more cells, containing each one or two nuclei. The nuclei sometimes appear in the living condition as clearer spaces, sometimes as darker spots. The colour of these bodies in life is greyish with a slightly greenish or brownish tinge. My notion of these bodies was that they came really from the solutions of salt or sodium citrate used in the dissections. Quite recently examining the blood of a rudd (Leuciscus erythrophthalmus) for trypanosomes, in Norfolk, I was surprised to find a quite typical example of these organisms. In this case the blood, in which no trypanosomes could be found, had been taken from the heart of a fish by means of a capillary glass tube, which had been previously washed through with normal saline solution. Hence there was a possibility that the body seen might have come from the salt solution. It may, however, be some form of organism inhabiting blood. I content myself with noting the occurrence of these bodies fairly frequently in tsetse-fly preparations.

In one fly I noted oval, refringent, motionless bodies in the proctodæum. In the sucking stomach I noted, in the wall, "long, greenish-yellow filaments, consisting each of a row of joints of varying lengths."[1]

Nomenclature of the structural parts of trypanosomes.—There is much diversity in the names applied by different authors to some parts of the trypanosome body, more especially with regard to the smaller chromatic nucleus, and also in the use of the terms anterior and posterior. The smaller nucleus is termed usually in England the micronucleus; in France the centrosome; and in Germany the blepharoplast. Each of these terms is open to objection, the term micronucleus, in my opinion, least of all, so long as it is used in a purely descriptive sense to mean simply a small nucleus; the danger is, however, that it leads to instituting a comparison with the micronucleus of Ciliata, which is a totally different structure, a reserve generative nucleus. Whatever the nature of the micronucleus of a trypanosome may be, there is no evidence that it is composed solely of generative chromatin.

The terms blepharoplast and centrosome raise the whole question of the nature of the bodies so named—a question upon which it is possible to have much difference of opinion. It is enough for me to refer here to the recent memoir by Goldschmidt and Popoff (**16**), in which the subject is discussed at length, and to state my own views: I regard a centrosome as an achromatic body, in connection with a nucleus; and a blepharoplast as a body of the same nature as a centrosome, but in connection with a protoplasmic locomotor apparatus, such as a flagellum or cilium. This is the sense in which I understand these terms; others may differ from me.

With regard to trypanosomes, I may point out, in the first place, that the "micronucleus" is certainly a chromatic body and cannot be classed with achromatic structures. Apart from its staining reactions, which are more intense than those of the

[1] Compare Stuhlmann (**41**, p. 47).

larger nucleus, I may cite the observations of Schaudinn (**40**), who describes it as arising in Trypanosoma noctuæ by an unequal division of the zygote nucleus; and I may further draw attention to the condition in Trypanoplasma, where it is as large as, or even larger than, the other nucleus, so that the term micronucleus for it becomes rather a misnomer.

In the second place, I may point out, as others have done also, that the flagellum does not arise from the smaller nucleus, but quite independently of it, from a minute basal granule.[1] The behaviour of the flagellum and of the smaller nucleus in division also shows clearly their complete structural independence, as I have pointed out below (p. 193).

I consider the basal granule of the flagellum as a true blepharoplast in the sense in which I have defined the term above; and I regard the nuclear apparatus of trypanosomes as specialised into two distinct portions, one regulating the function of locomotion, the other that of nutrition. Hence I consider that the terms kinetonucleus and trophonucleus, suggested by Woodcock (**42**), express most correctly the true nature of these bodies, and I shall employ these terms in my descriptions, though it is often convenient to speak of the trophonucleus simply as the nucleus, and as a further abbreviation I shall sometimes refer to the two bodies as *n* and *N* simply.

From the true blepharoplast arises the flagellum, which passes to the surface of the body and runs along the edge of the undulating membrane as the marginal flagellum, until it reaches the end of the body, where it becomes a free flagellum of greater or shorter length. According to Schaudinn's observations on the formation of the locomotion apparatus in Trypanosoma noctuæ (**40**), there exists also a distal blepharoplast, as it may be termed, situated at the

[1] Dutton, Todd, and Hannington (**15**, p. 219) point out that "the thickened end of the undulating membrane ends not in it [*n*] but in a pinkish basal granule or 'diplosome'" (why "diplosome"? It is only double when about to divide). Compare Novy (**33**), pp. 5 and 6.

free end of the flagellum. It is stated that the flagellum and undulating membrane are formed from the achromatic apparatus of a nucleus spindle, of which the central spindle gives rise to the flagellum and the mantle fibres to the myoneme-fibres of the undulating membrane, while the two centrosomes become the blepharoplast. These statements have received some confirmation from the observations of Robertson (**37**) on the formation of the flagellum in the trypanosome of Pontobdella muricata.

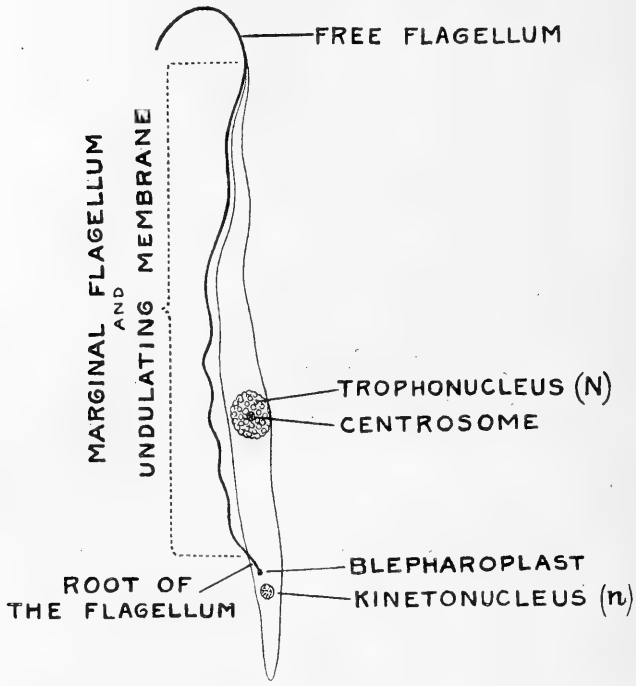
I have frequently noticed, especially in the slender forms of T. grayi, that the flagellum is distinctly thickened at the free extremity, but I should not like to affirm the existence of a definite blepharoplast at this point. Prowazek (**36**) has also described for T. lewisi a complicated arrangement of anchoring granules and fibrils. I can only say that in my material I have not seen them.

With regard to the use of the terms anterior and posterior much has been written, and it has been pointed out by Woodcock (**42**) and Lühe (**26**) that what is morphologically anterior in one trypanosome may be posterior in another. It cannot, I think, be disputed that there may be two entirely different lines of phylogenetic evolution amongst the organisms grouped generally as trypanosomes (see below, p. 219), but in the present state of knowledge it is not possible to state definitely which of the two possible modes of orientation is applicable to a given species. Hence it would be best, perhaps, to avoid altogether the use of the terms anterior and posterior in describing trypanosomes, and to speak of the flagellar and anti-flagellar extremities, but such a terminology becomes very cumbrous in practice. I shall speak of the flagellar extremity as anterior, the anti-flagellar as posterior, using the terms in a purely descriptive sense and without prejudice to the morphological questions involved. So far as I have observed, when trypanosomes are moving freely they travel usually with the flagellar extremity directed forwards, but when pushing their way amongst blood-corpuscles they do so with the free flagellum of the undulating

membrane directed backwards, their movement in the latter case being similar to that of a Trypanoplasma.

The accompanying diagram (Text-fig. A) is intended to

TEXT-FIG. A.



make clear the terms and the use which I shall make of them in this memoir.[1]

II. OBSERVATIONS ON TRYPANOSOMA GAMBIENSE.

(A) T. gambiense in the Blood of the Vertebrate Host.

My observations relate chiefly to T. gambiense in the blood of monkeys, all of which had been infected, directly or indirectly, by inoculation with cerebro-spinal fluid from sleeping sickness patients, with the exception of one, which became infected by the bites of tsetse-flies caught in the

[1] See also Appendix (p. 252).

neighbourhood of Entebbe; I shall refer to this monkey briefly as the fresh-fly monkey. I have also preparations of cerebro-spinal fluid from patients, and of the blood of a chimpanzee, eighteen days after inoculation with human cerebro-spinal fluid. I have no preparations of human blood showing trypanosomes.

In all cases alike the trypanosomes are distinguishable by their general build and appearance into slender, stout, and intermediate forms.[1] In the living condition the slender forms appear more snake-like, and are active in their movements, while the stout forms are fish-like in shape, and less motile. In successful preparations of blood-smears the three forms can usually be distinguished readily, especially in films fixed with osmic acid vapour, which preserves the body-form very perfectly. In films preserved by the ordinary drying method the different forms appear less differentiated. This is evidently the result of a slight flattening-out of the body, produced by the method of drying. The two extremes of the series—namely, the stout and slender types—contrast with each other, not only in their proportions, but also in the relative length of the free flagellum, which is long in the slender forms, short in the stout ones. The trypanosomes of the intermediate type can also be subdivided into two types by this character, so that all the trypanosomes in a given blood-preparation can be divided into two classes—those with long and those with short flagellum. This character, which is plainly seen in the figures of Bruce and Nabarro (**5**), is more reliable for use as a morphological distinction than the stoutness of the body—a character liable to alteration or deformation due to imperfect preservation.

The extreme of the stout type is seen in the so-called

[1] Moore and Breinl (**30**) state that they are unable to distinguish any marked dimorphism, and that the so-called males and females are "arbitrarily chosen" extremes in a continuous series. It was never pretended that they were anything else than the extreme differentiations, obvious naturally, of a neutral or intermediate type; as will be described below, after twenty-four hours in the invertebrate host the intermediate forms disappear, and only the extremes remain, arbitrarily selected or differentiated by the action of their environment.

"stumpy" forms (figs. 9, 10, 17, 20, 21, 33–35), in which the body is short and stout, usually rounded off abruptly at the posterior end, and with a very short flagellum. These stumpy forms are not always to be found, but when present are unmistakable. I found the greatest contrast in the trypanosomes from cerebro-spinal fluid (figs. 30–35), in which the slender and the stumpy forms recall the two types of Trypanosoma dimorphon, and very few intermediate forms were to be found. A count made from four smears of cerebro-spinal fluid gave twenty long and slender forms (43·47 per cent.), eighteen short and stumpy forms (39·12 per cent.), of which one was dividing, and eight intermediate forms (17·39 per cent.). In two preparations, made from the blood of the same monkey (478) on two successive days (pp. 230, 231), I find on one day (figs. 1–5) no stumpy forms, though they were present in smears made the next day (figs. 6–10); and it is noteworthy that in flies fed on this monkey on the first day I obtained no development of the trypanosomes, while the batch of flies fed two days later showed a normal type of infection (p. 231). My best infection of T. gambiense in the tsetse-fly was from a batch of flies fed on monkey 478 on a day (Oct. 19th, p. 237) when it was showing trypanosomes, few in number, but sharply differentiated in form (figs. 11–13).

Castellani (**13**) has stated that T. gambiense, i. e. the trypanosome of Gambia fever, moves with the flagellum forwards, that of the sleeping sickness with the blunt end forwards. I find that the trypanosomes I have observed push their way through blood-corpuscles with the blunt end forwards, but when moving freely they tend to travel with the flagellum forwards.

With due allowance for variations in form and size, I find the structure of T. gambiense very uniform. The nucleus is large and lodged near the middle of the body; it appears to be a compact mass of chromatic granules, without any definite limiting membrane. Near the nucleus a few coarse granulations are commonly seen, which may, however, be few in number or absent. The kinetonucleus is usually close to

the posterior end, but may be a short distance from it when the posterior end is prolonged into a point. The kinetonucleus is usually a minute round body, but may be rod-shaped, a form possibly connected with impending division, though this seems to me doubtful. I observed that in slender forms the kinetonucleus, if rod-shaped, was placed transversely (figs. 12, 30), but in stumpy forms the rod-shaped kinetonucleus was applied to the surface of the body and had a more longitudinal direction (figs. 34, 35). In good preparations it can always be seen clearly that the flagellum does not arise directly from the kinetonucleus, but from a minute granule, which, as stated above, I regard as the true blepharoplast. In some cases the blepharoplast may be very close to the kinetonucleus, or may be over or under it in the preparation, giving the impression that the flagellum arises directly from the kinetonucleus. The marginal flagellum stands well off from the body, forming an undulating membrane of some depth when seen in profile. The distinction between body and undulating membrane is sharp in osmic preparations, but less so in films fixed by drying, owing, I believe, to the deformation and flattening out of the body produced by the latter method. The flagellum takes, in fixed preparations, a variable number of turns, corresponding to pleats in the undulating membrane, which depend probably on the movements of the animal at the time when it was fixed; but as a rule the membrane is least pleated in the extreme forms, both slender and stumpy (compare figs. 5 and 13), most so in the intermediate form. The anterior end of the body is prolonged into a slender filament, running parallel to the flagellum, sometimes nearly to the end of it.

Most previous observers have described and figured T. gambiense as being vacuolated (see especially Bruce and Nabarro [**5**] and Castellani [**13**]). It has been regarded as almost a normal feature of the species to have a vacuole near the kinetonucleus.[1] In my experience these vacuolated

[1] "Für besonders charakteristisch wird von einiger Forschern eine Vakuole gehalten, die . . . . nicht immer in gleicher Deutlichkeit hervortritt," Lühe (**26**), p. 115.

forms are very rare. I have found them only in trypanosomes from the cerebro-spinal fluid, never in those from the blood, and in the former they are by no means universal. I consider the vacuolated condition as an abnormal one, due to the parasite being in unfavourable conditions, or to the reaction upon it of a diseased enfeebled host. Bruce, Nabarro, and Greig (**6**, p. 20) express the view that trypanosomes do not find cerebro-spinal fluid so favourable for growth as blood, and are stunted; they also note (loc. cit., p. 32) post-mortem forms, with large vacuoles, and deformed. Plimmer (**35**) regards the vacuolated form as "probably due to some condition of environment."

Prowazek (**36**) has given three figures of T. gambiense (named by him T. castellanii), all of which differ a good deal from the appearances I am accustomed to see. His first figure (loc. cit., fig. 108) looks like a stumpy form with a vacuole, but it has a long free flagellum, and is perhaps an ordinary form deformed. His second figure (loc. cit., fig. 109) shows a long slender form, evidently in process of division, as *n* and *N* are divided; that being so, one looks at once for the split flagellum which would naturally be found at this stage. The author has figured in the cytoplasm a slender filament which I identify, without hesitation, as the daughter flagellum not traced for its whole length. I think this figure throws some light on the fibrils Prowazek introduces into so many of his figures of trypanosomes. The author's third figure shows a trypanosome "with long, narrow nucleus." Never in all the many trypanosomes of this species that have come under my observation have I seen one with a nucleus such as Prowazek figures in this specimen; I am inclined to regard it as one in which the nucleus has become deformed in the process of smearing.

In short, I can but state that Prowazek's figures represent forms which, in my experience, are aberrant and abnormal; whether this is to be explained by the influence of technique on the parasites, or of a European climate on their hosts, I leave an open question. I am convinced, however, that the appearance, and even the structure, of trypanosomes may be greatly affected by the condition of their hosts.

I am also unable to make head or tail of the "atypical" forms described by Castellani (**13**). I have never seen anything like them. I note that he states that they occur especially in the last stages of disease. As my object was to trace the normal life-history of the parasite, I avoided as much as possible all material in which the parasites were likely to be influenced by a sickly condition of the host. Perhaps that accounts for the great discrepancy between my observations and those of these investigators with regard to the vacuolation of the trypanosome.

(B) The Development of Trypanosoma gambiense in Glossina palpalis and other Diptera.

My observations and experiments on the fate of T. gambiense in invertebrate hosts were carried out chiefly on Glossina palpalis, but I also made a few observations on the species of Stomoxys, common at Entebbe, and on the two common Entebbe mosquitoes, one a species of Tæniorhynchus, the other of Mansonia.

The results obtained with Glossina palpalis were remarkably uniform, and will be briefly summarised before entering into full detail. The trypanosomes at first multiply in the digestive tract of the fly, and by twenty-four hours are found to be differentiated into two types, slender and stout, sharply distinct from one another, with no intermediate forms. The next day, that is, at about forty-eight hours, these two types are succeeded by a more uniform type, so far as structure is concerned, but varying from slender to fairly stout, with all possible transitions, and of considerable length. On the third day after infection trypanosomes are always to be found in the digestive tract, and are forms of great length, relatively, varying from slender to stout, sometimes appearing degenerate in structure and diminished in number, but in other cases numerous, active, and with no signs of degeneration. On the fourth day trypanosomes are very rarely to be found, and, if present, are very scanty in number and of large

size, but are usually absent altogether. In no case have I found any signs of T. gambiense in the tsetse-fly later than the fourth day after infection. Nabarro and Greig (**31**) found T. gambiense in G. palpalis up to seventy-one hours after feeding; animal trypanosomes up to 100 hours. Throughout these four days of development T. gambiense undergoes a steady and well-marked increase of size.

In the other Diptera I found that T. gambiense went through the same changes of form and structure as in Glossina palpalis. In Stomoxys, however, no trypanosomes were found on the second day (forty-eight hours) after infection. My mosquito experiments are very incomplete, but I found active trypanosomes in Tæniorhynchus as late as seventy-two hours after infection.

In spite of much searching T. gambiense was never found in any organs except those in which digestion of the blood was proceeding; that is to say in the stomach and intestine.

I will now proceed to a more detailed description of my observations.

Preparations made from flies shortly after infection (July 31st, p. 228, and Sept. 8th, p. 229) show the gradual disappearance of the trypanosomes of intermediate type by their conversion partly into slender forms, but chiefly into stout forms. I think it is safe to assume that the intermediate forms with long flagellum become converted into slender forms (compare figs. 41–44), while those with short flagellum become stout forms. There are also many dividing forms found at this period, indicating active multiplication. The process of differentiation is complete twelve hours after infection, and then we have the two sharply marked types, slender and stout, which characterise the first day after infection. Seen in the living condition the slender, twenty-four-hour forms appear transparent, of serpentine appearance, and very active; the stout forms are fish-like or whale-like in form, opaque and granular, and sluggish in movement (figs. 102, 103).

The slender forms (figs. 45, 48, 52, 56–58, 61, 62, and P. R. S., B 78, Pl. XII, figs. 1–6) are distinguished in prepa-

rations, not only by their snake-like form, but also by the clearness of their cytoplasm, which is free from granulations, and remains scarcely, or not at all, tinged by the Romanowsky method of staining. Comparison of earlier stages show that these slender forms arise from the slender or intermediate forms met with in the blood before it is taken up by the fly, by absorption of the granules in the cytoplasm. The whole appearance of these forms suggests the activity and mobility which they are seen to possess when observed in the living state. The flagellum is very long and stands well off from the body, but the undulating membrane is not greatly pleated. The kinetonucleus is a circular dot usually placed at or very near the posterior end, but sometimes at a short distance from it. The nucleus has a dense, compact appearance, and usually a compressed form, but very frequently an irregular outline, with sometimes an appearance as if portions of chromatin were being detached from it, as noted in my preliminary communication (fig. 56, and P. R. S., B 78, Pl. XII, figs. 4, 5, and 6).

The stout forms (figs. 46, 47, 49–51, 53–55, 60, 63–65, 76, and P. R. S., B 78, Pl. XII, figs. 7–14), on the other hand, are large and obese, with cytoplasm coarsely granular and staining deeply. The flagellum is short, the undulating membrane but slightly pleated, and the kinetonucleus, which is circular or rod-shaped, is usually some distance from the posterior end, a point in which they contrast with the "stumpy" forms found in the vertebrate body. Sometimes the posterior end is prolonged into a short "rostrum," a feature which becomes exaggerated at a later stage. The nucleus is large and loose in texture, but of definite outline, often with a peripheral ring of coarser granules, but never showing the appearance of chromatin being ejected, as in the slender forms.

Not only do these two forms of trypanosomes differ in structure and appearance, they also show a curious difference in the mode of division. When the slender forms divide the two daughter-kinetonuclei keep close together at the posterior

end (fig. 217; see also P. R. S., B 78, Pl. XII, fig. 6); but in the stout forms, when the division has reached a certain stage, one of the two daughter-kinetonuclei passes forwards and takes up a position between the two daughter-nuclei, thus producing a characteristic division-stage (compare figs. 54, 55, and P. R. S., B 78, Pl. XII, fig. 12).

On the second day, that is, about forty-eight hours after infection, the trypanosomes are seen to be changing into a type which reaches its perfection on the third day, and is best described from its later development. What I will call the third-day type of trypanosome is of considerable length, appearing under forms both slender and stout, but with transitions between these two variations (figs. 83–96). The body is generally cylindrical, tapering gradually anteriorly and bluntly rounded off posteriorly. The kinetonucleus is round or rod-shaped, sometimes large, and generally situated some distance from the posterior end. The undulating membrane is not much pleated, and the free flagellum is short, even in the more slender forms (fig. 86).

In the preparations of about forty-eight hours we find this type sometimes fully perfected, sometimes only beginning to make its appearance. In one fly I found in the red blood forms similar to those described above as characteristic of twenty-four hours after infection (figs. 76, 77), while the black blood showed forms more advanced towards the third-day type (figs. 73–75). The question at once arises, how does this change of type come about? On account of the uniformity of structure shown by the third-day type I am inclined to derive them all from the stouter type seen at twenty-four hours, and to regard the more slender forms seen on the third day as derived by divisions from the stouter forms. In that case what becomes of the remarkable slender forms seen at twenty-four hours? It would be a tempting hypothesis to suppose that they have conjugated with the stout forms, and that the big forms of forty-eight and seventy-two hours represent zygotes, but I am unable to bring forward any facts in support of this supposition. If the stout twenty-four-hour

forms give rise to all those found later, it is implied that the slender twenty-four-hour forms die off; it is, of course, possible that they may persist and give rise to the later slender forms, and I must confess that my observations do not enable me to decide this point with certainty.

In my smears of flies dissected on the third day, I find great differences in the condition of the trypanosomes. In those from one batch (Oct. 18th, p. 235) the trypanosomes were almost without exception excessively granular and frequently also very vacuolated. They gave me the impression of being degenerate forms, with impaired vitality (figs. 82*a*–88). In another batch (Oct. 22nd, p. 237), however, I found in my smears abundant healthy-looking trypanosomes (figs. 89–92), not vacuolated nor excessively granular, and in the living condition they were extremely active, so much so that I thought I had to do with T. grayi.

In both cases alike, however, no trypanosomes were to be found in the flies dissected the day following.

On the fourth day (ninety-six hours or so) I have very rarely found trypanosomes present in the fly, and only once in smears of this period (Sept. 12th, p. 230). My preparations of them are, unfortunately, very poor, but it is at least possible to trace the form and features of the trypanosomes, which are of a large type, differing in no essential particular from those of the day before (figs. 97–101).

In no case did I ever find T. gambiense in the fly after the fourth day.

The disappearance of T. gambiense from the gut of the tsetse-fly, on or after the fourth day after infection, may mean either that the trypanosomes die out completely, or that they pass into some form which has not been recognised. If they really die out in the fly without completing any life-cycle, it would indicate, in my opinion, that Glossina palpalis is not the true host for this trypanosome, and that some other invertebrate host must be sought for it. I discuss this question more fully below. If, however, the disappearance of the trypanosomes is only apparent, and they really

persist, there are many possibilities which suggest themselves. First, they might pass into some other organs of the fly; against this I may urge that I have repeatedly examined all organs of the fly likely to harbour trypanosomes at various periods after infection, and never found a trypanosome or anything that suggested a stage of a trypanosome, outside the digestive tract. Secondly, they might assume some minute ultra-microscopic form; in that case, however, the function in the life-cycle of such a form would almost certainly be that of infecting a new vertebrate host by inoculation, and every experiment to produce infection with Glossina palpalis more than forty-eight hours after the fly had infected itself gave negative results. A third possibility is suggested to me by my observations on the encystation of T. grayi; it is possible that the trypanosomes which disappeared from the stomach and intestine passed on into the proctodæum in order to become encysted there. It is a matter of the deepest regret to me that I did not make smears of the proctodæum, but the idea of such a possible development of the trypanosomes was not present in my mind when I was in Entebbe; since I never saw T. gambiense in the proctodæum, I did not make any preparations of this region, and so the golden opportunity of deciding this point was lost to me.

Dutton, Todd, and Hannington (**15**) have published observations on the fate of T. gambiense after being taken up by various Arthropods. In G. palpalis they find "unaltered parasites in the alimentary canal up to forty-eight hours; living but altered trypanosomes up to seventy-two hours after feeding." This does not agree with my experience; I find the trypanosomes beginning to alter in character a few hours after feeding; by alteration, however, the authors mean, apparently, coarse or violent modification of trypanosome-structure. In Stomoxys trypanosomes were found up to twenty hours after feeding. In the larva of Auchmeromyia luteola they were found up to twelve hours after feeding; in Anopheles up to forty-two hours. Trypanosomes were

also obtained in a louse. There is nothing that calls for notice in their observations except the "rounded forms," which they believe to "arise from the englobation of single trypanosomes which have cast off blepharoplast and undulating membrane and become spherical." The authors describe their formation more in detail in the rat flea (louse?), in which they note "granular, indistinct, obviously degenerating parasites." I regard the rounded form seen by Dutton, Todd, and Hannington as trypanosomes succumbing to, and being digested by, the digestive juices of the alimentary canal, and quite distinct from the rounded forms described by Koch (**20**) and Stuhlmann (**41**), which probably form part of a true developmental cycle. I have not found rounded forms of T. gambiense in any of my preparations.

III. Observations upon Trypanosoma grayi.

My material of this trypanosome is limited, because, for reasons explained above, I did not systematically search for it in Glossina palpalis, so that all the instances of its occurrence that came under my ken were in flies used by me for studying the changes undergone by T. gambiense. In this way I found in all six flies containing T. grayi. Fortunately this trypanosome, when it occurs at all, is found in such vast swarms that a single infected fly furnishes an abundant material. Hence, I have been able to discover some facts of interest relating to this form, notably the process of encystation, not previously observed, so far as I am aware, in any trypanosome, and I have also studied carefully the distribution and occurrence of the many forms of this trypanosome in the different regions of the intestine of the fly; but with regard to this latter point, it is to be regretted that my material is defective, inasmuch as none of my five flies had any trypanosomes in the proventriculus, so that I have had no opportunity of studying the forms occurring in this part of the digestive tract. In spite of much searching I have never found this trypanosome in any organs of the fly other than

the digestive tract (stomach, intestine, and proctodæum). Of the six flies infected by T. grayi that I studied, in one case (Nov. 2nd, p. 238) I did not make any notes, unfortunately, as to the exact provenance of the trypanosomes. With regard to the other five, one (Nov. 10th, 1st fly, p. 241) had the trypanosomes only in the proctodæum. A second fly (Nov. 10th, 2nd fly, p. 241) had evidently not fed recently, and contained only a small quantity of black blood in the intestine, and trypanosomes were found only in the black blood and the proctodæum. The remaining three flies (Nov. 13th, p. 243, Nov. 14th, p. 244, and Oct. 10th, p. 232) showed trypanosomes swarming through the red blood (stomach), black blood (intestine), and proctodæum. In my preliminary account of the encystation (P. R. S., B 79, p. 35) I stated that it was rare to find them in the proctodæum, but I now recognise that this was a mistaken impression on my part; indeed, my limited experience indicates rather that the trypanosomes of this species always occur in the proctodæum, even when they are absent in other parts. But I have never found T. gambiense in the proctodæum.

While I was at Entebbe I had little time to draw and study accurately my preparations, but I made a few drawings of my slides from one fly (Oct. 10th), and some of them were published in the preliminary report by Gray, Tulloch, and myself (P. R. S., B 78, Pl. XIII, figs. 23 and 28). I have since then examined my slides of this fly much more carefully, and found that in this case also the proctodæum contained vast swarms of the trypanosome (p. 233), a fact which I had overlooked at the time of writing my report.

Trypanosoma grayi occurs under such a bewildering variety of forms and sizes that it is a matter of great difficulty at first to see any order or regularity in them. This difficulty is increased by the fact that in one fly the trypanosomes will be found reaching a much larger size, as a whole, than in another; thus, in the flies of Nov. 10th (2), Nov. 14th, and Oct. 10th, some of the trypanosomes are of very large proportions, while in the fly of Nov. 13th they are much smaller and more

slender in build. From my observations I have come to the conclusion that all the different forms may be grouped in three classes, the first of which has many subordinate subdivisions. I distinguish the three classes recognised by me in the following manner: (1) Ordinary or multiplicative forms; (2) slender forms; (3) Herpetomonas forms.

(1) The ordinary forms occur in a variety of sizes due principally to their growth and multiplication. We may distinguish, in the first place, adult or full-grown forms—those of the largest size. These are elongated forms, with the body more or less cylindrical in build; in some the body tapers anteriorly to a fine point (figs. 140, 158, 173, 218, etc.); in others, on the contrary, the body diminishes in thickness very gradually towards the anterior end, up to a point at a short distance from the extremity, from which it narrows rapidly to a stumpy point (figs. 138, 139, etc.). We may conveniently distinguish these two forms as the serpentine and the vermiform respectively; the free flagellum is short in both, but appears longer in the serpentine form, perhaps, in some cases, on account of the difficulty in distinguishing the exact anterior termination of the body. A third variety of the large forms is the tadpole form, in which the posterior end of the body is swollen out (figs. 142, 221, etc., and P. R. S., B 78, Pl. XIII, figs. 23, 26); these are the forms which I formally identified as females, but I am now inclined to regard them simply as full-grown forms which are about to multiply by division.

The process of division, which will shortly be described in detail, leads to a small daughter-individual being split off from the larger parent-individual; in this way young forms are produced (fig. 220, etc.)—the smallest individuals of the ordinary type. These young forms are always present, and frequently very abundant (Nov. 14th, p. 244). Between them and the largest forms every gradation of size is to be found; hence we may safely assume that the small daughter-forms produced by division grow up in time into the large forms, unless they develop into some other type, as will be described presently.

To sum up, we may classify the ordinary type of T. grayi roughly into serpentine, vermiform, tadpole-like, young, and intermediate (i. e. growing) forms. To these, however, must be added a sixth form, the significance of which is not at all clear to me. These are the round forms frequently present, and obviously connected with the young forms. It is easy, when round forms occur, to trace a series of transitional forms between them and the young forms, but whether round forms become young forms, or vice-versâ, it is not so easy to determine. A typical round form has a more or less spherical body, giving off a flagellum, which is entirely free except at its origin (fig. 136). Gray has figured a further development of the round forms, in which the flagellum becomes wrapped round the body in such a way as to simulate a cyst or cuticle in optical section (P. R. S., B 78, Pl. XIV, figs. 44–48), and also forms in which the flagellum appears to be entirely lost (loc. cit., figs. 49–51). I have not found such forms in my preparations, but I have seen those that my colleague has figured. According to Koch (**19**) and Stuhlmann (**41**), such forms are a regular part of the life-cycle, and Robertson (**37**) also describes them in the trypanosome of Pontobdella. Koch describes them as arising by the break-up of large, multinuclear forms, possibly zygotes. Robertson, on the other hand, considers them to be the first form assumed by trypanosomes when taken up from the blood of the vertebrate. Both Koch and Robertson describe the formation of small forms from the round forms.

As round forms are not very frequent in my preparations, I am not able to make any definite statements about them, except that they are connected by transitions with small forms. I am inclined to regard them as a normal temporary resting phase of the small forms, though in a few cases, perhaps, they may be due to imperfect fixation or other deformation due to technique—for instance, alterations during the dissection of the fly before fixation, when owing to any cause the making of smears has been delayed.

In all the different varieties of the ordinary form, as I have

called it, of T. grayi, the structure of the body is in general very uniform, though showing great variations in some points. As regards the cytoplasm, I find remarkable differences, which are evidently due to differences in the action of the stain used (Giemsa's mixture); two types of staining are produced which are well exemplified in the series of dividing forms figured by me (figs. 204–216), all of which are drawn from two preparations made from smears of the same blood, and stained in Giemsa's solution side by side in the same dish. In one preparation the cytoplasm is bluish in colour and shows coarse granulations deeply stained, while the flagella are very faintly stained, appearing sharp and delicate, often difficult to make out clearly (figs. 204, 207, 212, 213, 215, 216). In the other preparation the cytoplasm is reddish in tint and the granulations are scarcely seen, or not at all, while the flagella are deeply stained and appear thick and very distinct (figs. 205, 206, 208–211, 214). The differences in the staining are probably due to differences in the use of the Tannin solution, used to differentiate the stain (see p. 169).

The nucleus (*N*) of T. grayi presents itself as a clump of granules, sometimes compact, oval or round, and in the latter case sometimes showing a distinct rosette-like structure (fig. 182, and P. R. S., B 78, Pl. XIII, figs. 35, 39); more often the nucleus is quite irregular in shape and loose in texture, and in one fly (Nov. 10th, [2], p. 241) the nucleus shows streaks of granules apparently trailing irregularly out from it, in nearly all the large forms (figs. 138–140). This suggests that the coarse granulations of the cytoplasm represent chromidia derived from the nucleus; but they stain slightly differently with Giemsa's stain, the granules of the nucleus having a redder tint, while the chromidial granules are more purple, like the kinetonucleus in tint.

The kinetonucleus (*n*) is large and rod-shaped, its length being about twice its breadth; it is superficial in position, and often appears to bulge from the surface of the body. In very thin smears it is often torn out of the body. It is very

compact and stains deeply, so that no structure can be made out in it.

The most remarkable variations can be seen in the relative positions of n and N. I distinguish six types[1]: (1) n well in front of N, with distinct space between (figs. 181, 182); (2) n just in front of N, with no space between (fig. 156); (3) n is at the side of, or overlapping N (fig. 175); (4) n just behind N (fig. 176); (5) n far behind N (fig. 160); and (6) n terminal (fig. 152). Miss E. Y. Thomson has made some counts for me of the different types, which I record below:

		(1)	(2)	(3)	(4)	(5)	(6)
Nov. 13. Red blood	Numbers	1	30	45	47	320	7
(figs. 151–155, p. 243)	Percentage	0·22	6·66	10	10·4	71·11	1·5
Nov. 13. Black blood	Numbers	28	145	73	132	346	—
(figs. 156–164, p. 243)	Percentage	3·8	20	10	18·2	47·7	—
Nov. 13. Proctodæum	Numbers	11	55	33	62	179	5
(figs. 165–170, p. 244)	Percentage	3·1	15·9	9·56	17·9	51·5	1·4
Nov. 10. (2) Black blood	Numbers	113	332	168	65	17	—
(figs. 138–143, p. 241)	Percentage	16·3	47·9	24·1	9·3	2·4	—
Nov. 14. Red blood	Numbers	8	158	108	48	42	—
(figs. 171–173, p. 245)	Percentage	2·19	43·4	29·67	13·18	11·53	—
Nov. 14. Red-black blood	Numbers	40	209	863	314	207	—
(figs. 174–176, p. 245)	Percentage	2·4	12·8	52·8	19·2	12·6	—
Nov. 14. Black blood	Numbers	678	8	155	20	50	—
(figs. 177–182, p. 245)	Percentage	74·4	0·8	17	2·1	5·4	—
Oct. 10. Black blood	Numbers	14	56	49	14	6	—
(figs. 226*a*–231, p. 233)	Percentage	10	40·3	35·2	10	4·3	—
Oct. 10. Red blood	Numbers	25	44	39	3	3	—
(figs. 218–226, p. 232)	Percentage	21·9	38·5	34·2	2·6	2·6	—

These figures show in one fly (Nov. 13th) a great preponderance of forms with n posterior to N, or even terminal, especially in the red blood; but in all others the forms with n in front of or close beside N greatly preponderate. (In these statistics no account is taken of the slender and the Herpe-

[1] A convenient graphic notation from these types is as follows:

(1) $\frac{n}{N}$; (2) $\overset{n}{N}$; (3) n/N; (4) $\frac{N}{n}$; (5) $\frac{N}{n}$; (6) $\left|\begin{matrix}N\\n\end{matrix}\right.$. A type with n terminal but anterior could be written $\left|\begin{matrix}n\\N\end{matrix}\right.$

tomonas-like forms, in which *n* is invariably in front of *N*.) The forms with *n* posterior or terminal are interesting, as it is very probable that they represent very nearly the form under which T. grayi occurs in the vertebrate host; and in this connection their tendency to preponderate in the red blood is significant. I recognise now as erroneous my former statement (P. R. S., B 78, p. 243) to the effect that the forms with *n* posterior were nuper parentes, though this may be so in a few cases (see p. 192). It may be said, however, that for the forms occurring in the intestine of the fly, the normal and typical position of *n* is a little in front of *N*, for not only is this the condition found in those forms (the slender form and the Herpetomonas form presently to be described) in which the position does not vary, but it is also the position which *n* always assumes when division is about to commence.

I have made a detailed study of the process of division in T. grayi, with the object of being able to distinguish clearly between the stages of multiplication and of conjugation. The process of division shows some variations; I will first describe what appears to be the most usual course of events, and after that I will deal with the deviations from this course that are met with.

The first event in the process of division is that the minute basal granule or blepharoplast (see p. 172) of the flagellum divides into two (fig. 151). At the same time the kinetonucleus becomes slightly enlarged and elongated. Starting from the two blepharoplasts the flagellum splits longitudinally and as the two minute blepharoplasts are at first connected by a delicate thread, we obtain a common and characteristic early stage of division, in which, in a trypanosome otherwise of normal aspect, the flagellum shows the appearance of a loop at its proximal end (figs. 204, 205). The splitting of the flagellum continues and the two blepharoplasts travel away from each other, so that the appearance of a loop is lost; but at this stage there is nearly always seen a crossing over of the two halves of the flagellum in the region where it is

split in two (figs. 206, 207). This crossing of the flagella marks a second characteristic stage during which the division of the kinetonucleus takes place. The splitting of the flagellum proceeds a certain distance and then breaks off, so that a longer and a shorter flagellum result from it (figs. 208–210). About the time the splitting of the flagellum is complete the nucleus divides. I have found stages of the division in which a thread or band ran between the two daughter-nuclei, connecting two granules, doubtless of centrosomic nature, imbedded each in the mass of one of the two daughter-nuclei, and difficult to distinguish from the chromatin granules surrounding them (figs. 209, 210). As may be seen from the figures, there is the utmost diversity in the relative positions of the two nuclei and the two kinetonuclei. I must therefore retract my former statement (P. R. S., B 78, p. 243) as to the constant position of n and N after division. When the division of the nucleus and flagellum is complete, the body begins to split (figs. 209–212), starting from the anterior extremity between the two flagella. The body always divides into two unequal portions, giving rise to individuals which are markedly unequal in size and may be distinguished conveniently as parent and daughter respectively. The parent takes the longer flagellum, that is to say, the principle portion of the original flagellum; the daughter takes the shorter flagellum. The two individuals are attached last in the neighbourhood of the kinetonucleus (fig. 212; compare fig. 217), until they finally break loose.

By some authors it has been stated that, in the division of trypanosomes, the daughter flagellum is not split off from the parent, but grows out independently of it. I have paid much attention to this point in T. grayi, and can find nothing to indicate that the new flagellum is formed otherwise than I have stated above, namely, by a process of splitting which starts from the division of the blepharoplast. At the same time I can quite well imagine that in other species the process may take a different course. It is seen that even in T. grayi the splitting does not extend to the whole length

of the parent flagellum. In another species the amount of splitting might be much less, or might not extend beyond the division of the blepharoplast. I do not wish, at present at least, to extend my statements as to the splitting of the flagellum beyond the case which I have studied. That the flagellum does actually split in T. grayi is shown, I think, by the fact that in the early stages the daughter flagellum is distinctly finer and more delicate than the parent flagellum (figs. 205–208). I see no reason why this should be so if the daughter flagellum grew out independently from the blepharoplast.

The process of division described in the foregoing paragraphs is that most usually found, and represents, I believe, the normal course of events. The commonest departure from this course is seen in the division of the flagellum, which may be hastened and be completed before the division of the nucleus (fig. 213), or even before that of the kinetonucleus (fig. 215). This variation shows clearly that the flagellum is independent of the kinetonucleus, a fact which, in my opinion, is a further indication that the kinetonucleus should not be confounded with a true blepharoplast or centrosome.

(2) The slender type (figs. 144, 145, 172, 224–226, etc.), is very uniform in its characters and exhibits but slight variation in contrast with the many forms which I have grouped together as the "ordinary" type. The body is elongated and slender; the cytoplasm is free from coarse granules as a rule, but occasionally a few are seen in the posterior part of the body; N is compact, sometimes very compressed; n is large, rod-shaped or round, generally filling up the whole width of the body, and invariably in front of N; the flagellum is distinct and stains deeply; it stands off but little from the body, forming a very shallow undulating membrane, which is scarcely or not at all pleated, and the free flagellum is very long, and often appears distinctly thickened at its free termination.

The chief variation exhibited by this type is seen in the degree of slenderness of the body; usually slender, with a

compressed nucleus (figs. 225, 226), it is sometimes stouter, with a round or oval nucleus, thus showing an approach to the ordinary young forms (fig. 224, etc.). In one instance (Nov. 10th, 1st fly, p. 241) I have found such moderately slender forms connected by transitions with round forms (figs. 132–137). Only in a single instance have I found what appears to be division; but I am by no means certain that this is not really a case of two trypanosomes accidentally superposed (fig. 147).

The slender type is distinguished in life by its extreme motility. The trypanosomes are seen darting across the field of the microscope in the manner aptly termed "en flèche" by French writers. The body is held stiff and moves with the flagellum, vibrating rapidly in advance. These are the forms termed by Novy (**32**) and by Gray and myself (**29**) "male" forms; I shall consider the point more in detail presently.

As a special development of the slender type should be reckoned, perhaps, the immensely elongated forms seen in figs. 242–244. Unfortunately I have not noted the region in which they occur. A similar form has been figured by Gray (P. R. S., B 78, Pl. XIII, fig. 33). My first impression of these remarkable forms was that they were simply spermatozoa of the fly, but they are connected by transitions with more ordinary forms.

(3) The Herpetomonas-type is to be regarded as a special modification of the slender type, though probably originating directly, in some cases, from ordinary young forms. The H-forms (as I may term them for brevity's sake) are minute and very slender, with the chief bulk of the body behind the kinetonucleus, in front of which the body tapers rapidly to a filamentous prolongation, sometimes of considerable length (figs. 163, 164, 169, 170, 184, 185, 187, 188, etc.). Posteriorly the body is usually bluntly pointed. The nucleus has the form of a clump of granules about the middle of the body. The kinetonucleus is invariably in front of the nucleus. The cytoplasm is clear, but sometimes contains a few coarse

granulations, which frequently occur in front of the kinetonucleus. The undulating membrane is sometimes distinct (fig. 163), but as a rule is rudimentary or absent. The flagellum is very long and very slender and delicate; it stains feebly or not at all, and is often difficult to trace. A typical example of this type is unmistakable, and contrasts sharply in many points with the slender type described above.

The H-forms have been found by me becoming encysted in great numbers in the proctodæum of one of my flies (Nov. 14th, p. 246). I have two smears of this specimen; one of them is a thin smear, the other much thicker. Both the smears show all stages of the encystment in abundance, but in the thin smear the cyst-wall, when developed, is nearly always more or less damaged, being evidently of soft consistence. In the thick smear the cyst-wall is usually intact and perfect, but the finer details of the contained body are not so well shown. Figs. 186–197, 202, 203 are drawn from the thin smear, as were also the figures published by me in my preliminary communication to the Royal Society (P. R. S., B 79, p. 37). Figs. 198–201 are from the thicker smear, which I had not examined at the time of making my preliminary communication.

My preparations of the proctodæum show, in the first place, a certain number of young forms of the ordinary type (figs. 183, 186). Similar young forms are also found as the preponderating type of trypanosome in the hinder part of the intestine of the same fly (p. 245), a fact of interest as indicating that the H-type arises from the young forms of the ordinary type. In the second place the smears show a large number of the H-forms (figs. 187, 188), which are also found sparingly in the hinder part of the intestine. In life they were observed swimming freely, and also occurring in masses attached to the wall of the proctodæum. In the third place there are the various stages of encystment, from the earliest modification of the H-form to the ripe cyst in its final form (figs. 189–202). The cysts at all stages were free in the lumen of the proctodæum, and not attached in any way to its walls.

The first sign of encystment is a shortening of the flagellum, which at the same time appears to become thicker and more distinct, an effect perhaps largely due, however, to its being difficult to distinguish the exact limits of the flagellum from the slender anterior prolongation of the body (compare figs. 189, 192). While the flagellum is becoming retracted the cyst-wall begins to appear round the hinder end of the body (figs. 189–193) in the form of a granular secretion, which stains a dull red with Giemsa's stain. The rate at which the cyst is formed shows great variations relatively to the retractation of the flagellum. In some, with the flagellum still long, an abundance of the cyst secretion is seen (fig. 190). In others, with the flagellum almost completely retracted, the cyst is only just beginning to be formed (fig. 193). The substance of the cyst-wall appears to be made up of distinct masses or grains of the red-staining substance, between which is a more fluid matrix, and appearances are often obtained very similar to those figured by Prowazek for Herpetomonas muscæ-domesticæ (**35***a*). The term "Schleim-cysten," applied by Prowazek to the cysts of Herpetomonas, appears suitable for the present case also, as the frequency with which burst or damaged cysts are met with (figs. 192, 195, etc.) indicates that the substance of the cyst-envelope is of a soft nature.

While the cyst-wall is being secreted the retractation of the flagellum is proceeding, until all that can be seen of it is a round or oval red-staining mass, connected by a red streak with the blepharoplast (figs. 194, 195). The appearances seen at this stage (which is of very common occurrence) are remarkable, and suggest strongly the "flagellar vacuole" described by Leishman as giving rise to the flagellum in the flagellated culture-form of Leishmania. Here, however, the flagellar vacuole, if it may be so termed, is, in its relation to the flagellum, the inverse of that of Leishmania—that is to say, it does not precede its formation but results from its retractation. The flagellar vacuole disappears and only the streak is left (fig. 196); finally the streak fades away too, and

the retractation of the flagellum is complete. Meanwhile the cyst-wall, which made its appearance as a cap round the posterior end of the body, is secreted more and more towards the anterior end, and when the flagellum has completely disappeared, the cyst is formed round the anterior termination of the body, thus producing the characteristic pear-shaped cysts which are very abundant in the preparation (fig. 198). This appears from Prowazek's description to be the final stage of the cyst in the case of Herpetomonas; not so in T. grayi. The cysts, at first pear-shaped, with unlike ends, become more oval, with ends alike or scarcely distinguishable (figs. 199, 200), and finally they become more or less circular in outline, with the wall of even thickness all round (fig. 202). This stage is apparently the ripe cyst, and the last stage that can be observed in the body of the fly.

While the process of encystation is going on, noteworthy changes are taking place in the nuclei. The trophonucleus becomes resolved into chromidia. Usually one large, irregular mass of chromatin can be seen, together with a variable number of irregularly scattered chromatin grains (fig. 197). At the same time the kinetonucleus diminishes in size, apparently also as the result of fragmentation. Amongst the chromatic grains scattered in the body, some can be distinguished by their reddish colour, like that of the nucleus, others by their purple colour, like that of the kinetonucleus (fig. 195); the latter are fewer in number. It is important to note that the cyst substance also stains a reddish tint, similar to that shown by the granules of the nucleus, and it is possible that the disruption of the nucleus is in relation to the secretion of the cyst.

In the ripe cysts the kinetonucleus seems to disappear completely. In some of them it can still be made out plainly (fig. 202). In others it cannot be identified with certainty, but amongst the chromatic grains some can be seen which exhibit a more purple tinge than the others, and which represent probably the kinetonucleus broken up. The significance of these changes could only be made out by studying

the germination of the cyst, and that I have not been able to do.

In a few rare cases I have observed division within the cyst; one such case is figured (fig. 203).

Having, in the foregoing, described the various forms under which I have met with Trypanosoma grayi in Glossina palpalis, I propose now to discuss the significance of the different forms and their relations to one another. This is not an easy matter in the incomplete state of our knowledge of the life-cycle of this trypanosome. Moreover, the forms which T. grayi assumes are so different that they might well be taken for different species were it not that they are all linked by transitions. Taking first the forms which I have grouped together as the "ordinary" type, we may, I think, regard them as the multiplicative form of the trypanosome in the fly, that is to say, the form which, when taken from the vertebrate host, has the function of feeding, growing, and multiplying by division to produce the vast swarms of trypanosomes which are found in infected flies. I base this opinion on the following grounds: First, the large size which this form reaches, a character not likely to be exhibited by forms destined to pass back from the fly to a vertebrate host; such forms are more likely to be of small size; secondly, the frequent occurrence of division and production of young and intermediate forms; thirdly, the great variability of this form, especially in the position of the kinetonucleus. Far from being constantly in front of the nucleus, the kinetonucleus may be behind it or even terminal. From our knowledge of other trypanosomes, I think we may assume that the forms with *n* terminal probably represent most nearly the form under which T. grayi occurs in the vertebrate host. It is true that it is not even known that this trypanosome has a vertebrate host. I have elsewhere (P. R. S., B 79, p. 38) brought forward arguments to favour the belief that T. grayi has a vertebrate host, and is not simply a parasite of tsetse-flies, and in my opinion the occurrence of

a form with *n* terminal, like an ordinary blood-trypanosome, is still more in favour of this belief. I would add, further, that from the conditions under which T. grayi was found to occur in one of my flies (Oct. 10th, p. 232), I am of opinion that the host of T. grayi is a bird, probably some of the numerous species of diving birds which swarm on the shore of the Victoria Nyanza.

We have next to consider the slender forms, formerly regarded by me as male. I do not think this interpretation can be maintained, in view of their distribution and mode of occurrence in the fly. Thus, in one of my flies (Nov. 10th, [1], p. 241), only slender forms, round forms, and H-forms occur, and are confined to the proctodæum (figs. 132–137). Some of them are fairly stout and approach young or ordinary forms in character, but with *n* constantly anterior to *N*. In Nov. 10th (2) also we find most typical slender forms in the proctodæum, but none in the intestine, where ordinary forms of all kinds occur (p. 242, figs. 138–150). In Nov. 13th we find no slender forms in the stomach, but in the intestine and proctodæum we find both slender forms and typical H-forms mixed with ordinary forms (figs. 156–170, p. 243). In Nov. 14th we find slender forms very sparingly in the stomach and intestine, while the proctodæum contains swarms of H-forms and cysts (figs. 171–216, p. 245). Finally, in Oct. 10th we find slender forms present fairly commonly in the stomach and intestine, together with ordinary forms, but in the proctodæum we find immense numbers of the slender and H-forms, without any ordinary forms (figs. 218–233, p. 233).

The data given above show that the primary habitat of the slender type is the proctodæum, of which it may constitute the sole trypanosome-fauna; and further that all other forms may be absent altogether from the fly. This, I think, sufficiently disposes of the notion that the slender form represents the male type. From the proctodæum both the slender form and the H-form may extend forwards; it is noteworthy, however, that the slender form gets further forward, being found in the stomach, while the H-form does not, so far as my

observations extend, get further forward than the hinder part of the intestine. It would be interesting to know if the slender forms penetrate as far as the proventriculus. Unfortunately none of the flies examined by me had trypanosomes in the proventriculus.

The slender form and the H-form are in all probability to be regarded as propagative forms, that is to say, as forms destined to spread the species to other hosts. In favour of this view may be urged their small size and great activity, the absence of multiplication amongst them, their mode of occurrence in the fly, and their constant structure, far removed from that of the ordinary blood-trypanosome and more approaching the Herpetomonas or Crithidia type of structure, that is to say, the type of structure usually associated, in these organisms, with parasitism in the gut of insects. The H-form becomes encysted in the proctodæum, and the cysts are doubtless destined to pass out with the fæces. What is the destiny of the slender type? The manner in which it extends forwards in the gut suggests strongly that it may be destined to infect fresh hosts by the inoculative method.

To sum up my conclusions as to the various forms of Trypanosoma grayi: They may be subdivided at the outset (using Doflein's appropriate terms) into (1) multiplicative forms, varying greatly in size and structure; and (2) propagative forms, very constant in both respects, but of two types. One of the propagative types terminates its lodgment in the fly by becoming encysted in the hind gut; the other is perhaps destined to pass forwards through the proboscis.

If the two types hitherto regarded as male and female be not in reality such, the question arises whether any of the numerous forms of T. grayi are to be regarded as sexual. It is usual, when slender and stout forms of trypanosomes occur together, to interpret them as male and female; but the only final and conclusive proof of such forms being sexual in nature is to find them conjugating. I have spent much time trying to find stages of conjugation in my slides of T. grayi,

but among the many thousands of trypanosomes that have passed under my vision I have found none that could be interpreted with certainty as conjugating forms, and very few which could be suspected of this. Since conjugating forms might conceivably be confused with dividing forms, I studied in detail, and have described above, the process of division and its variations, which are chiefly variations in the rapidity, relative to one another, with which the flagellum and the two nuclei (*n* and *N*) divide. I have found a few specimens which might perhaps be conjugating forms, and have figured three of them. In one (fig. 214) it is seen that the two flagella are widely separate, that there are two large kinetonuclei in close contact, and that there is a single compact nucleus, very dense in texture. If this be a variation of division, it is a remarkable one, not only in the very precocious division of the flagellum, and in the fact that the body has begun to divide before the nucleus has divided, but, above all, in the very large size of the two kinetonuclei; I have never seen any division-stage in which the daughter-kinetonuclei were of such large size. On the other hand, this specimen would very well bear interpretation as a fusion, which had commenced from the hinder end of two individuals, in which the bodies are nearly fused, the nuclei completely so, the kinetonuclei are beginning to unite, and the flagella are still quite separate. If this interpretation be the right one, fig. 216 might possibly represent a further stage of the same process. Another possible instance of conjugation is seen in fig. 143, in which the kinetonuclei are united, while the nuclei are distinct. If these three figures really represent instances of conjugation,[1] they do not throw much light on the characters of the males and females, except to show that one

[1] The forms figured by Stuhlmann (**41**) and interpreted by him as conjugation-stages are very different in appearance, especially in having the two flagella on the outer sides, furthest from each other, instead of close together. Stuhlmann considers the position of the flagella diagnostic of conjugation as compared with division; this makes it still more doubtful if the forms figured by me are really conjugation-stages.

partner has a long free flagellum, the other a short one. I am inclined to identify the two varieties of the ordinary form, termed above the serpentine and the vermiform types, as male and female respectively, and fig. 143 supports this view; but it must be confessed the evidence is meagre. Some of the large ordinary forms (figs. 138, 139) might then be really zygotes.

A few words, in conclusion, upon the differences between T. grayi and T. gambiense and the nature of the former. As a rule the two forms can be distinguished at a glance, whether in the living condition or in preparations. T. gambiense is sluggish in its movements, and when observed in vitro seldom moves out of the field. T. grayi, on the contrary, moves with great rapidity, and is very difficult to keep in the field. The slender forms, in particular, dart across like arrows. But in two flies containing T. gambiense seventy-two hours after infection, I observed that the trypanosomes were very active in their movements (Oct. 22nd, p. 237), and in the preparations very large trypanosomes were found.

The most constant distinctive feature of T. grayi is its large rod-shaped kinetonucleus. In T. gambiense n may be round or rod-shaped, but is always much smaller than in T. grayi. Moreover, in T. grayi, though the position of n is variable, it is most usually at the side of, or in front of, N, and when division is about to take place, n is always in front of N. In T. gambiense, so far as I have observed, n is always behind N by at least half the distance between N and the posterior end of the body. It is sometimes difficult to draw clear distinctions in words, but I think the figures show that it is impossible to confound T. grayi and T. gambiense at any stage. It is, nevertheless, interesting to note that in the two instances in which T. gambiense did not show any signs of degeneration on the third day in the fly, it made some approach to T. grayi in its appearance and activities. T. grayi, as seen in the fly, is probably a good deal different from the same species in the blood of the vertebrate host.

I think there can be no doubt that the forms described by

Koch (**20**) as developmental stages of T. gambiense were really T. grayi or a similar form.

With regard to the origin of T. grayi, our first notion was, as I have stated, that it was identical with T. gambiense; when this notion was dispelled I was at first inclined to regard it, with Novy, as a parasite of the fly itself, and my discovery of the encystation seemed to confirm this idea, which, however, more mature reflections, especially considerations of the habits of the fly, made me give up. In my preliminary report (P.R.S., B 79, pp. 38, 39) I have stated my reasons for believing that T. grayi has a vertebrate host, and I have nothing to add to them. For various reasons we suspected T. grayi to be an avian trypanosome, though we were not able to prove this, but Novy has shown that it is often impossible to find trypanosomes in bird's blood microscopically when their presence can be demonstrated culturally. The fact that a fly (Oct. 10th, 1905, p. 232), bred in captivity, becomes infected with T. grayi after feeding on fowls used to feed freshly caught flies, seems to me proof positive that T. grayi is an avian parasite [1]; but if so, I may point out as a corollary, it also suggests that T. grayi can be transmitted by the inoculative method.

IV. Remarks on the Life-cycle and Mode of Transmission of Trypanosomes.

The scientific study of the transmission of trypanosomes, and their relation to disease, dates from the publication of Bruce's masterly reports on his investigations upon Nagana in Zululand in 1895 (**3**, **4**). It is not necessary for me to dilate at length upon the results, well known to everyone, of these researches, admirable alike for their conception, execution and presentation, and marking, as Koch has well said, the beginning of one of the most important epochs in the study of the Protozoa.

The publication of Bruce's reports seems to have aroused almost immediately suspicions as to the true nature of sleep-

[1] Koch seems inclined to consider the crocodile as the vertebrate host of T. grayi or of other tsetse-fly trypanosomes.

ing sickness, since Blanchard (1) quotes a sentence from a memoir published in 1898, by J. Brault, in which the opinion is expressed that sleeping sickness is a protozoal disease caused by trypanosomes and transmitted by tsetse-flies. The news, however, of the discovery of trypanosomes in the cerebro-spinal fluid of sleeping-sickness patients, by Castellani in 1903, produced quite a chorus of prophetic utterances, predicting or arguing that sleeping sickness would prove to be transmitted by tsetse-flies. According to Blanchard, this view was expressed by himself on June 18th, by Brumpt on June 27th, and by Sambon on July 1st, 1903, in each case independently of the others. Let who will, however, claim the gift of prophecy or the talent for drawing reasonable inferences from the analogy of established truths, it was nevertheless Bruce, who, in collaboration with his colleagues of the Sleeping Sickness Commission, Nabarro and Greig, first supplied the experimental demonstration of the transmission of sleeping sickness by tsetse-flies. All later observations have but confirmed the statements of these pioneer investigators, without so far adding anything of importance to their results. That Glossina palpalis can and does convey the infection of sleeping sickness, may, I think, be taken as an established fact; and up to the present no other method of infection has been proved to exist. This brings the etiology of sleeping sickness into line with that of other trypanosome infections, all of which, so far as present knowledge extends, are transmitted only by the intermediary of blood-sucking invertebrates, with a single exception—the well-known case, namely, of dourine in horses, a disease known to be transmitted from sick to healthy animals by coitus. This exception to the general rule is of considerable interest, as showing that, in this case at least, the trypanosomes have the power of passing through mucous membranes.

There remains, however, the question of the exact manner in which the infection is transmitted by means of the blood-sucking invertebrate. In considering this question, the facts

known with regard to malaria rise at once to the mind. The researches of Ross, Grassi, and others have demonstrated that in malaria the mosquito is a true host in which the parasite goes through a complicated developmental cycle, at the end of which, and not before, the mosquito is able to infect a fresh vertebrate host with the parasite. A mode of infection of this kind may be termed conveniently a cyclical type of infection, and, since it is effected by the mosquito inoculating healthy subjects with the parasites, we may further characterise it as the inoculative cyclical type. It is an obvious suggestion, from the analogy of malaria, that trypanosomes may also undergo a cycle of development in their invertebrate hosts, a suggestion that received, apparently, concrete proof in the well-known investigations of Schaudinn (**40**) upon Trypanosoma noctuæ. But it is becoming increasingly evident, I think I may say, that Schaudinn's statements with regard to Trypanosoma noctuæ must be regarded with scepticism until they have received confirmation, in view of the many possible sources of error from mixed infections which his material presented. Nevertheless, Schaudinn's results have been regarded by many as conclusive proofs that trypanosomes pass through a developmental cycle in their invertebrate hosts. I am conscious myself of having gone to Uganda to investigate this question with a distinct bias in my mind, believing that the infection of sleeping sickness would prove to be of a similar type to that known in the case of the malarial parasite, and stated by Schaudinn to occur in T. noctuæ—namely, the cyclical inoculative type.

On the other hand, many experimental results, so far obtained, indicate that the mode of transmission of sleeping sickness, and perhaps of other trypanosome infections also, is not of the type of malaria, but is in many cases, at least, a direct one—that is to say, that the trypanosome does not go through a developmental cycle in the invertebrate host, but is inoculated mechanically by the proboscis of the blood-sucking intermediary. Thus Brumpt, in 1903 ([7] p. 1497), expressed the view that the rôle of the tsetse-fly could not

be compared to that of Anopheles in malaria, since the tsetse transmits only for forty-eight hours, and no longer. At the present time authoritative opinion on the subject may be said to be divided into two camps, some denying the existence of cyclical infection, others believing it to occur. An extreme example of the former school of thought is Novy, who is of the opinion that the insect plays but "a passive or mechanical part" in the transmission of trypanosomes ([**33**] p. 13 of reprint), and who has cast grave but reasonable doubts upon the correctness of Schaudinn's famous investigations upon Trypanosoma noctuæ.

Confining our attention, for the moment, to the transmission of sleeping sickness, that is to say, of T. gambiense by Glossina palpalis, we have, in the first place, the transmission experiments carried out by Bruce, Nabarro, and Greig (**6**), which can be most conveniently summarised in a tabular form:

Number and reference of experiment.	Number of flies fed on patient.	Flies fed on healthy monkey.	Interval between the two feeds.	Infection produced after—
114 (loc. cit., p. 57) . .	880	530	8 hours	65 days
115 (loc. cit., p. 58) . .	881	509	8 ,,	65 ,,
99 (loc. cit., p. 59) . .	582	508	24 ,,	70 ,,
97 (loc. cit., p. 60) . .	294	255	24 ,,	48 ,,
116 (loc. cit., p. 61) . .	354	267	48 ,,	65 ,,

These experiments prove the transmission of the trypanosome of sleeping sickness by G. palpalis, and indicate that the transmission is a direct one, since with a longer interval than forty-eight hours no infection was obtained. I say "indicate," because the fact that freshly-caught flies were used for the experiments invalidates conclusions as to the exact method of the infection, since it was also proved by the same investigators that freshly-caught flies may produce

infection with trypanosomes, without having been fed previously upon infected animals. Thus, in one case a monkey became infected when 216 freshly-caught flies had been fed on it in batches over a period of fifteen days (loc. cit., p. 62); in a second case 894 flies produced an infection after twenty-eight days (loc. cit., p. 62); and in a third case, 759 flies infected after twenty-three days (loc. cit., p. 63). To these three cases may be added two experiments recorded by Greig and Gray (**18**); in the first 980 flies produced an infection after forty-six days (loc. cit., p. 106); in the second, 2299 flies were fed on a monkey over a long period without infecting it. Finally, reference may be made to the result obtained by us (**29**, p. 245), in which a batch of 134 flies fed on a monkey (as a control experiment) produced an infection in it. If we add all these results together we find that a total of 5282 tsetse-flies, freshly caught in the neighbourhood of Entebbe, infected five animals; but if we take separately the experiments of Bruce, Nabarro, and Greig (**6**), made at a time when remedial measures had not been undertaken at Entebbe, a much higher average is the result, since we find that 1869 freshly-caught flies produced three infections, an average of one infection for 623 flies, which is a number very little higher than that of the flies which were operative in the eight-hour transmission experiments. There was, therefore, the possibility that the infections produced in the transmission experiments tabulated above were not really produced by transmission from the infected to the healthy subject, but by flies which had been in an infective condition when first caught, before being used for the experiments. It is noteworthy also how much more quickly, in all the successful experiments, the infection was produced with the freshly-caught flies, so that it would appear to be almost more dangerous to be bitten by free flies in a sleeping sickness locality than by those artificially infected in the laboratory.

A further point to note in the experiments tabulated above is the relatively large number of flies required to produce

infection at the short intervals (eight hours). I believe this is to be accounted for as follows: when a batch of flies has been fed and is put on a second animal eight hours later, the majority of flies being gorged with blood do not feed a second time; the flies that feed at the second opportunity are probably those which did not feed on the first occasion. It is comparatively rare for a fly not to gorge itself the first time it is put on to an animal.[1]

For these reasons we sought to obtain evidence more decisive as to the exact nature of the transmission. As stated in our preliminary report (**29**, p. 244), we took one fly at a time, fed it partly on the infected animal, and then transferred it to the healthy animal. In this way we infected[2] nine out of ten animals each with a single fly, which may, I think, be taken as conclusive proof of the transmission being direct, and not due to any pre-existing infectiveness of the flies used, since the proportion of infective flies amongst those caught while near Entebbe was at that time probably less than one in a thousand. It is true that these experiments were done with the "Jinja" strain of trypanosome (a further proof that it was not due to any previous infection of the flies used in the experiment); but I think it may be claimed that they prove what they were intended to prove—namely, that the tsetse-fly can, and does, effect direct mechanical transference of

[1] I have not made detailed reference to the experiments of Dutton, Todd, and Hannington (**15**), as I wish to confine myself more especially to the region in which I worked. The investigators named seem to have been singularly unsuccessful in their experiments, only four (three with T. gambiense, one with T. dimorphon) giving a positive result out of a total of thirty-nine. It is noteworthy that in three out of their four successful experiments the animal that became infected had been bitten by Glossina fusca as well as G. palpalis. Cazalbou (**14**) seems to have been more fortunate in his experiments with freshly caught G. palpalis. Dutton, Todd, and Hannington consider it "certain that . . . mechanical transmission cannot be the only way in which Trypanosoma gambiense is transmitted from man to man" (loc. cit., p. 213).

[2] Thus confirming a suggestion of Bruce (**4**, p. 3) who wrote, with reference to nagana, "I have no doubt one fly would give the disease if taken while feeding on an affected animal and placed straightway on a healthy one."

trypanosomes from an infected to a clean animal by means of its proboscis.

To prove direct transmission, however, does not disprove the existence of an indirect or cyclical method. It is logically impossible to prove a universal negative since any such proposition is invalidated by a single instance to the contrary. We can only say that our attempts to obtain evidence of a cyclical infection gave negative results. Such attempts were of two kinds: first, experiments to show periodicity in the effectiveness of the flies, such as is known to occur in the case of malaria; secondly, observations on the fate of T. gambiense when taken up by the fly. By experiment we obtained infection up to, but never after, forty-eight hours from the time that the fly fled on the infected animal. By observation, as already stated at length in this memoir, it was found that T. gambiense at first multiplied and became differentiated in the gut of Glossina palpalis or other Diptera, and also increased continually in size, but sooner or later died out and could never be found in G. palpalis on or after the fifth, sometimes not on the fourth day after infection. In all forty-two tsetse-flies have been examined by me at ninety-six hours or later after feeding on infected animals, with invariably negative results, although all the flies from the same batches were found to contain T. gambiense more or less abundantly when dissected at earlier periods.

Very different results from those obtained with sleeping sickness are furnished by the recorded experiments and observations with regard to nagana, the tsetse-fly disease of animals caused by T. brucii. If we take first of all Bruce's experiments on the transmission (3) we are struck at once by the much smaller number of flies used to produce a result. Where hundreds were required for an infection with sleeping sickness, tens or even units were sufficient for an infection with nagana. Thus in one experiment (228, loc. cit., p. 5) eight flies, fed four times on a healthy dog immediately after feeding on an infected one, produced

an infection; the same result was attained in another case (Experiment 222, loc. cit., p. 5) with eight flies after only three feeds; with twenty-four hours' interval (Experiment 291, p. 6), nine flies produced an infection after thirty-eight days, and again, with the same interval (Experiment 232, p. 6), twelve flies infected a dog in the same number of days; with forty-eight hours' interval (Experiment 317, loc. cit., p. 7) seventy flies fed in batches over thirty-one days were required, but longer intervals were not successful. With freshly-caught flies, one hundred and twenty-nine flies, fed in batches, infected a horse after twenty-four days (Experiment 225, loc. cit., p. 15), and ninety-eight flies infected a dog after nineteen days (Experiment 236, p. 16). If these experiments favour the view that the infection is a direct one, they at least indicate that infection of this type is much more easily obtained in this way with T. brucii than with T. gambiense. There is, however, in one of Bruce's experiments a circumstance which, taken in connection with an experimental result of ours, seems to me to show clearly that the infection of T. brucii was not always of the direct type. Bruce's Experiment 225 (loc. cit., p. 15), to which reference has been made already, was carried out to answer the question, "Is the tsetse-fly capable of giving rise to the disease if taken out of the fly country into a healthy locality?" In the account given we read that "the method of carrying out this experiment was to go down to the fly country in the early morning, catch the flies, return to the top of the Ubombo, and straightway place them on the animal under experiment. The greatest care was taken that the flies were caught on a perfectly healthy animal, as to have allowed them to puncture one already affected by the disease would naturally vitiate the experiment." Now in our experiments on direct transmission, already recorded (p. 244), we found that if the fly, after feeding on an infected animal, were fed on two healthy animals in succession, only the first healthy animal became infected, not the second—that is to say, that by puncturing the skin of a healthy animal the

proboscis is "cleaned" for a second one. Hence, if the infection of T. brucii were only by the direct method, the flies caught off a healthy animal, in Bruce's experiment, should have been non-infective. The experiment seems to me, therefore, to indicate that in the case of T. brucii there is infection of a type other than the direct—that is to say, that cyclical infection occurs, doubtless in addition to direct infection.[1]

Turning from experiment to observation, we have the results obtained by Koch (**19**) and Stuhlmann (**41**), who have studied the development of T. brucii in Glossina fusca, and have found a state of things which are in sharp contrast with my results for T. gambiense and G. palpalis.[2]

Koch (**19**) found a multiplication of the trypanosomes, with differentiation into stout and slender forms; next, multinuclear forms, believed to be zygotes, from which arose round forms, which in their turn gave rise to small forms with *n* in front of *N*. Long narrow forms, with *n* far in front of *N*, were also found. In the proboscis fluid trypanosomes were found resembling blood-trypanosomes in form and size, in addition to other forms. Attempts to infect rats with the trypanosomes in the digestive tract of tsetses were always without result. Koch gives reasons for believing that the infection produced in the fly differs according to the state of the trypanosomes in the blood of the sick animal; in flies fed on animals freshly infected, with many trypanosomes in their

[1] The great infectiveness of tsetse-flies in the case of nagana is notorious. According to the unanimous testimony of travellers, confirmed by Bruce's observations, it is only necessary for susceptible animals, such as horses or cattle, to pass through the "fly-belts," in order to acquire the disease, practically as a certainty. This is not the case in sleeping sickness. In Uganda I was struck not so much by the number of sick persons in affected areas as by the number of healthy persons living under precisely the same conditions. The infection of sleeping sickness by the bites of tsetses may be compared to a lottery, in which the prizes are few and the blanks are many.

[2] Bruce (**4**) found, in tsetses infected with nagana, trypanosomes in the stomach up to the end of the fifth day, so long as any blood remained undigested.

blood, development was not obtained, but in flies fed on sickly animals with few trypanosomes in their blood, a vigorous growth and development of the trypanosomes resulted.

Stuhlmann (**41**) has published a preliminary account of the results obtained by himself and Kudicke. These investigators worked both with freshly-caught flies and with flies bred in captivity. The latter were found to become most easily infected if fed on the infected animal for their first meal after being hatched from the pupa; in 80–90 per cent. of the flies so treated, after a short time (2–4 days) the intestine (Hinterdarm) became full of trypanosomes, indifferent forms showing many division-stages. Stuhlmann's figures of sections show the trypanosomes present in enormous numbers, similar, in my experience, to an infection with T. grayi. From the intestine the infection was found to spread forwards into the stomach (Mitteldarm), but rarely extended further forward than this region, unless the flies were re-fed on healthy animals; when that was done the infection could be traced forward as far as the proventriculus. Infection of the proboscis was not produced in artificially infected flies, but was found in a small percentage of those naturally infected. It was found, moreover, that in artifically infected flies the infection produced in the intestine often gradually died out, and only about 10 per cent. of the experimental flies obtained an infection of the stomach and thoracic intestine; thus the percentage of flies that became thoroughly infected in the laboratory came to about the same as the percentage of flies found infected in nature. The authors figure forms which resemble to a certain extent some forms of T. grayi, but which are characterised by a small round kinetonucleus. They distinguish indifferent forms, long forms, and small forms. The long forms are found in the proventriculus and œsophagus, rarely in the proboscis. The small forms are seen, from the figure given (loc. cit., fig. 158 *a*), to be very similar to the small forms of T. grayi with *n* in front of *N* (fig. 182) except for the characteristic difference of the kinetonucleus. It is remarkable, therefore, that the authors should find the

small forms almost exclusively in the proboscis, seldom in the gut, even in its most anterior parts; for, as I have described above, the corresponding forms of T. grayi are, on the contrary, most abundant in the hindmost parts of the digestive tract. Amœboid forms, with flagella reduced or absent, are also described, and are stated to be present sometimes in great numbers, but only in hungry flies. They are regarded as resting phases, and perhaps correspond to the round forms of T. grayi, but I have never seen anything like the "giant amœboids, with 4–16 nuclei," figured by Stuhlmann (loc. cit., fig. 154).

The following is the scheme of development suggested by Stuhlmann: The infection begins by a multiplication of indifferent forms in the intestine. From this point they spread forwards as far as the proventriculus, where Stuhlmann has seen and figured (loc. cit., fig. 152 *a*—*g*) appearances which he believes to be conjugation. After conjugation, probably, are produced the small forms, which may be the infective forms which pass back into a new vertebrate host. The nature of the long, slender forms is doubtful; they appear simply to die out. In no cases were trypanosomes found in the end gut (proctodæum), nor in the salivary glands, nor in any organs of the fly except the proboscis and the digestive tract proper—that is, as far back as the end of the intestine.

The fact that Stuhlmann and Kudicke used only flies bred in the laboratory for their infection experiments may be taken as proof that the trypanosomes observed by them in these flies were truly stages of T. brucii. Stuhlmann is of opinion, and I agree with him, that trypanosomes are not transmitted by tsetse-flies to their posterity; in other words, that so-called germinative or hereditary infection does not occur.[1] It is evident, then, that T. brucii not only multiplies

[1] I cannot follow Novy (**32**) when he refers to the supposed occurrence of trypanosomes in tsetses that have never fed on animals; he even commits himself to the extraordinary statement that "only a very small percentage of biting insects ever feed on blood" (loc. cit., p. 406). I can only explain such an utterance on the part of an investigator so skilled and experienced by

but goes through a developmental cycle in G. fusca, ending with Herpetomonas-like forms, probably destined to infect vertebrate hosts. These results are entirely different from those obtained by me with T. gambiense in G. palpalis, where I found, without exception, that the trypanosomes did not survive after the fourth day, did not extend forwards into the proventriculus, and did not produce forms with *n* in front of *N*. As compared with T. grayi, the most noteworthy point of the development of T. brucii is that the "propagative" Herpetomonas-like forms occur only in the proboscis or most anterior part of the gut. In T. grayi, on the contrary, they are most abundant in the hindermost part of the gut, where they may become encysted. This difference between the two forms indicates that in T. brucii there is only probably inoculative infection, and no encystment or contaminative infection.

From the results, therefore, both of experiment and observation, it seems to me proved that T. brucii goes through a true developmental cycle in at least one species of the tsetse-flies that are agents in its transmission. Why, then,

his lack of acquaintance with the habits and life-history of tsetse-flies—a knowledge only to be acquired, of course, in Africa. From my own experience, I agree thoroughly with Stuhlmann (**41**, p. 44) that Glossina lives exclusively on blood and contains in its gut only organisms taken up with blood or derived from its mother. Our experience is that Glossina is a most greedy and rapacious bloodsucker; we have seen it feed readily on frogs, lizards, snakes, chameleons, crocodiles (compare Koch [**21**], p. iv), and birds, as well as mammals. On one occasion, a chameleon put into a fly-cage was at once attacked by them, and, being unable to defend itself, was so bitten that it died in twenty minutes from loss of blood. We also made many attempts to feed tsetses in other ways than with blood, but always without result (compare Stuhlmann, loc. cit., p. 44). On the other hand, there is not the slightest evidence that the tsetse can inherit trypanosomes from its parents (compare Stuhlmann, loc. cit., p. 70). Dutton, Todd, and Hannington (p. 202) hint at this possibility, solely from the far-fetched analogy of Piroplasma in ticks; but in this case it is known that the mother-tick passes on to each of her offspring a supply of undigested blood containing the stages of the parasite. It is not necessary to point out that Glossina differs totally in both habits and life-history from either ticks or mosquitoes.

has T. gambiense given only negative results as regards a developmental cycle? If T. brucii is capable of developing, T. gambiense should be capable of doing so also. I can only explain my repeated failures to obtain development by the hypothesis that G. palpalis is not capable (in Uganda, at least) of acting as the true host of T. gambiense, but only of transmitting it by the direct method. Sleeping sickness, as is well known, has only been introduced into Uganda in comparatively recent times, having apparently come from regions further west. The appalling results produced by the epidemic brought about the appointment of our Commission, which carried on its investigations in Entebbe—that is to say, in a region where great mortality was taking place and the danger was most urgent. But in and around Entebbe G. palpalis is the sole species of tsetse-fly occurring, and has, therefore, obtained the sole credit of transmitting the disease which corresponds with it exactly in distribution. It is very possible that if our Commission had carried on its investigations in a region were more than one species of tsetse occurs, it would have been found that other species were equally effective. Brumpt, in 1904 (**8**), gave reasons for believing G. fusca to be also active in transmitting sleeping sickness.[1] But more decisive on the point is the observation of Koch (**21**), who fed forty-two Glossinæ fuscæ and eleven G. tachinoides, all of them young forms bred in captivity, on rats infected with T. gambiense, with the result that ten to twelve days later eight fuscæ and three tachinoides were found to be infected with trypanosomes that must be identified with T. gambiense, in view of the fact that virgin flies, as we may conveniently call them, were used for the experiment. No such result was ever obtained by me with G. palpalis; and I think it is a sound deduction from the observation of Koch, collated with my own experience, that G. palpalis is

[1] Ross (**38**), however, obtained "entirely negative" results with G. fusca, but acknowledges that there was "not sufficient material in these experiments to come to any definite conclusion."

not a suitable host[1] for the development of T. gambiense, though efficient in transmitting this trypanosome mechanically, while in other species of tsetses T. gambiense is capable of passing through a developmental cycle[2] To find support for this conclusion it is not necessary to rely solely on the analogy of the well-known facts of the transmission of malaria and mosquitoes. The interesting studies of Brumpt (**9**, **10**, **11**) on the transmission of fish-trypanosomes by leeches have shown that for a given species of trypanosome there is what may be termed a right leech, and that other species of leeches are wrong ones. Thus, for T. granulosum of the eel the right leech, according to Brumpt, is a Hemiclepsis, in which the development is completed, while three others, namely, Calobdella punctata, Hirudo troctina, and Piscicola geometra, were wrong leeches, in which only a part of the development was passed through. There is no reason to suppose that in the case of trypanosomes the same intimate relation between host and parasite does not obtain that is known to occur in the case of other parasites, protozoan or metazoan.

Still less can I adhere to the peculiar view of Novy, that trypanosomes do not go through any life-cycle in their invertebrate hosts, but only a process of multiplication analogous to that seen in artificial cultures. Novy seems to regard the invertebrate host as nothing more, so far as trypanosomes are concerned, than a kind of flying culture-tube, an imitation of art by Nature; and, like some

[1] From the experiments of Bouet (**2**) and Roubaud (**39**) there is evidence for regarding G. palpalis as the true host for T. dimorphon.

[2] In his most recent report Koch (**22**) states that in "Glossinen" (presumably G. palpalis) several species of trypanosomes can be found; one of which, so far found five times, can be identified with T. gambiense. It is not stated, however, in what way its identity is established; to judge from Koch's report, which is far from explicit, the identification was made on morphological grounds, a very unsafe criterion. Koch further states that in two of his five cases the trypanosomes were found in the salivary glands—a state of things contrary to all previous recorded experience (compare Stuhlmann [**41**], p. 23); without further explanation of these statements, further comment is impossible.

other writers, he does not seem to realise the essential distinction between multiplication and development. We can, perhaps, see in this attitude towards the problem the predisposition of an accomplished bacteriologist unaccustomed to think zoologically, if I may use such an expression. He expresses the belief that the trypanosomes found in biting insects are harmless parasites of the fly (p. 406) "derived from plant juices, stagnant waters, etc." (p. 404). In speculating about things unknown it is surely safer to reason from established data than from unfounded hypothesis. At the present time true trypanosomes are only known to occur in the blood of vertebrates, and in the stomachs of insects which suck the blood of vertebrates; hence, it is reasonable to assume that the insects in question obtain their trypanosomes from the vertebrates. When trypanosomes have been found in plant juices or stagnant waters it will be time enough to speculate on the possibility of blood-sucking insects obtaining them from such sources.

Another remarkable fact, to which I would draw special attention, is that trypanosomes, taken up into the digestive tract of the fly, do not infect susceptible hosts if artificially inoculated into them. Bruce ([**4**], p. 5) first discovered this curious fact, and could only infect with T. brucii from the stomachs of tsetses if inoculated not more than half an hour [1] after the fly had infected itself; all inoculations at later periods gave negative results. This has been confirmed by many subsequent investigators of trypanosome development, for example, by Prowazek (**36**), Koch ([**19**], p. 14), Bouet ([**2**], p. 474), Gray and Tulloch (**17**), and ourselves (pp. 227, 234). I agree with the explanation, first suggested I believe by Manson,[2] of this fact, that the trypanosomes

[1] Since infection by the bite of the fly can be obtained up to forty-eight hours, the trypanosomes inoculated by the tsetse must be those which remain in the proboscis and do not pass into the digestive tract. Bruce (**4**) observed trypanosomes of nagana in the proboscis as late as forty-six hours after feeding, though rarely. This agrees with the experimental results.

[2] I am not able to give the exact reference.

in the digestive tract of the fly are developmental, and not merely multiplicative forms, and are not ripe, so to speak, for transference to the vertebrate host. Novy (**32**) considers this to be due to T. gambiense having died out in the flies, but this is certainly not so in our experiment (pp. 227, 234), in which case the fly's intestine contained numerous active T. gambiense. In the latter paper of Novy, MacNeal and Torrey (p. 262) it is stated that "the fact that ingested trypanosomes lose their virulence so rapidly in the stomachs of insects indicates a loss of functional activity, especially of the power of multiplication." This, again, is certainly not the case; multiplication of the trypanosomes proceeds continually in the gut of the insect.

In my opinion the evidence now accumulated (I may mention especially Brumpt [**10**] and Prowazek [**36**] in addition to the authorities already cited) is conclusive in favour both of the general statement, that trypanosomes undergo development, as distinct from multiplication, in invertebrate hosts, and of the more special proposition, that certain species do so in tsetse-flies. I wish now to offer a few suggestions upon the mode of their development and the nature of the invertebrate cycle, as it may be termed. The possibilities of trypanosome development are bound up with the question of their possible or probable phylogeny and course of evolution in the past.[1]

Many authorities have pointed out that trypanosomes may possibly, if not certainly, have two distinct ancestral origins. The first is from a Trypanoplasma-like ancestor in which the anterior flagellum has been lost; this is the "trypanosome with flagellum morphologically posterior"

[1] As I pointed out in the discussion on Hæmoflagellates before the British Medical Association at Exeter, where I have ventilated some of the views here expressed. My phylogenetic speculations were, however, put aside by Sir Patrick Manson, who expressed the view that where a parasite is now found in two hosts, for instance, a vertebrate and an arthropod, it was inherited by both of its hosts from their common ancestor (see 'Lancet,' September 7th,

of Léger (**24**). The second origin is from a Herpetomonas-like ancestor, in which the insertion of the flagellum becomes shifted backwards to form the undulating membrane; Léger's "trypanosome with the flagellum morphologically anterior." Woodcock (**42**) and Lühe (**26**) have gone so far as to base generic distinctions on this alleged difference of origin. For evidence bearing upon evolution in past times the zoologist turns naturally to the documents supplied by the records of development at the present time; and it must be acknowledged that so far there has been no development evidence whatever forthcoming in favour of a Trypanoplasma-like ancestor. No one has yet recorded a developmental stage of a trypanosome with two flagella, not even in those of fishes, which seem most likely to be allied to Trypanoplasma. On the other hand, Herpetomonas forms are common, if not invariable, in trypanosome development, and we may agree with Brumpt (**10**) and Léger (**24**) that in such cases this form certainly represents the ancestral form of these intestinal parasites before they became "sanguicolous." We may therefore look upon the Herpetomonas-form in the development of trypanosomes as a true recapitulative larval form, a type with which zoologists and embryologists are sufficiently familiar in the development of animals of all classes. For the present a Trypanoplasma-like ancestor most remain hypothetical until concrete evidence for it is forthcoming in given cases.

Léger (**24**), confining his speculations to the trypano-

1907, p. 707). As I pointed out at the time, this view offers some difficulties; first, in view of the palæontological facts, showing vertebrates and arthropods to have been distinct in Silurian epochs, if not earlier; secondly, in view of the fact that "Hæmoprotozoa" divide their parasitism sometimes between an arthropod (insect or arachnid) and a vertebrate, sometimes between a leech and a vertebrate. I may point out further that the invertebrate host of a Hæmoprotozoon is always one that sucks the blood of the vertebrate; it remains, therefore, to be explained how the transmission from host to host was effected at the ancestral period when, ex hypothesi, all hosts of a given parasite belonged to a single species.

somes with flagellum morphologically anterior, considers that the Herpetomonas ancestor was "a primitively intestinal or entero-cœlomic [1] parasite of an invertebrate," not necessarily of a blood-sucking one, in which their entire life-cycle took place; that when the invertebrate became blood-sucking in habit, its gut parasites found themselves in a nutrient medium in which they were able to multiply enormously and were thus prepared for life in vertebrate blood, into which they finally succeeded in passing by inoculation through the proboscis. Their primitive habitat is supposed to be shown by the fact that only non-sexual multiplication takes place in the vertebrate, and the invertebrate host is necessary for their sexual development—a statement which, it may be remarked, is by no means definitely proved, however probable, for trypanosomes.

Léger's theory is logical and complete; it only seems to me to present one flaw. If we consider the transmission of trypanosomes generally, we find that it does not always take place by the intermediary of an insect, but may be effected by a leech; and if we include Hæmosporidia, as Léger does in his theory, we then have to reckon with Arachnida as well, in some cases. In other words, the constant trypanosome host is the vertebrate; the inconstant host is the invertebrate. I propose, therefore, to consider the facts from the standpoint of an opposite hypothesis, namely, that the ancestors of trypanosomes were primitively parasites of the gut of vertebrates, like so many flagellates known to exist at present, and that from the gut they passed into the blood of the vertebrate and finally into the gut of the blood-sucking invertebrate. If we attempt to imagine and to reconstruct on this basis the successive stages in the evolution of trypanosome life-cycles, we should probably have the following series of events:

(1) The ancestral form, ex hypothesi, was a flagellate parasitic in the vertebrate gut, which doubtless was

[1] I am not quite sure that I understand the meaning of this adjective, though it has a familiar sound to the morphologist.

disseminated as such parasites are now; it formed resistent cysts in the gut which passed out with the fæces, were scattered abroad, and contaminated the food of fresh vertebrate hosts.

(2) When such a form succeeded in penetrating the intestinal wall and passing into the circulatory system, it found itself in a situation from which there was no escape or outlet by natural channels. Hence, if it did not at once come into relation with blood-sucking invertebrates, it could only have infected new hosts by coming back to the intestine of the vertebrate, becoming encysted there, and passing out with the fæces, as in (1). There is absolutely no evidence that any trypanosomes develop in this way; but a cycle of this kind has been described for Lankesterella ranarum by Hinze whose statements are usually, though perhaps not very logically, considered to be refuted by the observations of Siegel on the Hæmogregarina stepanowi of the tortoise.

(3) Our trypanosome in the vertebrate blood may be supposed to have been taken up sooner or later by a blood-sucking invertebrate, the digestive juices of which it succeeded in resisting. It has acquired now a channel of escape from the vertebrate blood and is no longer obliged to become encysted in the vertebrate gut. Becoming adapted to the invertebrate gut, where it finds the nutriment, namely blood, to which it was accustomed, it now forms in the invertebrate gut the cysts which it formerly produced in the vertebrate. The cysts pass out with the fæces, are spread abroad, and reinfect the vertebrate host by contamination. This is the condition which I believe to be represented by T. grayi, described above; though it is possible that in this case contaminative infection is combined with inoculative, definite proof of either being as yet lacking.

(4) The trypanosome having become thoroughly adapted to the invertebrate gut acquires the power of passing forwards till it reaches its proboscis, and becomes inoculated into the vertebrate host, thus establishing the commonly-occurring inoculative type of infection. Now intestinal cysts become unnecessary and cease to be produced.

It is not necessary for me to dwell upon a possible fifth stage realised in Piroplasma, where the parasite passes through two generations of the invertebrate host. Nothing of the sort is known to occur in the case of trypanosomes.

It is not possible to prove or demonstrate a phylogenetic theory. One can only consider how far it suits known facts or overcomes difficulties. My theory has only one advantage over Léger's—that of explaining away the difference in the invertebrate hosts in different cases. Parasitic flagellates are found in the gut of invertebrates as well as vertebrates. If special stress be laid on the occurrence of Herpetomonas in insects which do not suck blood, such as the house-fly, I may refer to Prowazek's speculations on this form, especially his interesting suggestion that the house-fly is descended from blood-sucking ancestors, which acquired the Herpetomonas from vertebrate blood, so that H. muscæ-domesticæ would represent a stranded and persistent larval stage, comparable to, for instance, the axolotl amongst higher animals.

I have suggested above, and in a former memoir (**28**), the possibility that contaminative infection, the commonest of all methods of infection amongst Protozoan parasites generally, may occur also in the case of trypanosomes infecting vertebrates, basing my suggestion upon the encystation observed by me in T. grayi. Encystation has not been observed in any other species of trypanosome, and with regard to T. brucii, the observation of Stuhlmann (**41**) noted above, that the Herpetomonas-forms are found almost exclusively in the proboscis of the tsetse, rather indicates, as I have already pointed out, that the stage in the life-history which tends to become encysted in the case of T. grayi, does not do so in the case of T. brucii, which would probably be similar, in this respect, to T. gambiense. Moreover, experimental evidence, so far as it exists, is against the occurrence of contaminative infection; in the case of nagana Bruce (**4**) experimented with food and water from localities where the disease is rife, but failed to produce infection with it. Bruce also injected fæces of flies into susceptible animals,

but without result; it is a pity the experiment was not tried of contaminating their food with the fæces. Hence the results to hand of observation and experiment, though they are very incomplete, indicate that the trypanosomes of the pathogenic group, such as T. brucii, belong to stage 4 of my hypothetical phylogeny. Nevertheless, if encystation occurs in one species of trypanosome it may occur in others.

Contaminative infection implies the infection of the vertebrate by way of the digestive tract. It is surprising how often the occurrence of accidental infections of this kind has been noted (see Laveran and Mesnil [**23**]) and yet how seldom it has been the object of direct experiment. Thus Bruce ([**8**], p. 46, Experiment 223) records the case of a dog which became infected with nagana after eating a piece of coagulated blood from the heart of a diseased heifer. It is usual to explain away such infections by supposing that an animal which becomes infected by way of the digestive tract must have had somewhere a lesion through which the trypanosomes penetrated, but this is pure assumption, and from the analogy of Dourine-infection it is quite as feasible to suppose that infection from the digestive tract can take place through the mucous membrane. If the trypanosomes can resist the digestive juices of insects, they may also resist those of vertebrates, and in this connection I may refer to the discovery by Léger (**25**) of an intestinal Trypanoplasma in the fish Box boops. All these facts seem to me to render perfectly possible and even probable the contaminative infection of the vertebrate, by way of the digestive tract, not merely as an exceptional occurrence, but as the normal course in those cases where, as in T. grayi, intestinal cysts are formed by the trypanosome. I may remark that if we start our phylogenetic deductions from Léger's hypothesis, it is not easy to explain the occurrence of encystation in T. grayi, unless we deny to it a vertebrate host altogether—a difficult position, it seems to me, in view of the habits of the fly.

The foregoing arguments may seem to many too specula-

tive and unpractical. Phylogenetic speculations may, however, have a practical value, if only in widening our point of view, and at the same time formulating the possibilities of development, for which the investigator should be prepared. I think the studies of trypanosome development have been too much dominated by preconceived assumptions, and that investigators have too often been biassed by analogies and predisposed to force new facts into old formulæ. It is not necessary to suppose that the development of trypanosomes should be in all cases of the same pattern. If it be true that amongst trypanosomes two quite distinct lines of evolution are comprised, we may expect to find the greatest differences in their mode of development.

In conclusion, I may say that with regard to the main problem of my investigations, namely, the life-cycle of T. gambiense, it is a matter of great regret to me that I have little but negative results to bring forward, and can only offer speculations and hypotheses where I had hoped to have contributed definitely established facts. It was my desire to return to my investigations on this subject, but owing to the lack of the necessary support I have been obliged to abandon the idea. I think now, however, for reasons given above, it would be better to study the etiology of sleeping sickness in regions where it is endemic and where other species of tsetses besides G. palpalis occur. In both these respects Uganda is probably less suitable for the study of the problem than the Congo.

The following propositions summarise briefly the conclusions or personal opinions to which my investigations or reflections have led me.

(1) In Uganda Trypanosoma gambiense begins, but does not complete, a developmental cycle in Glossina palpalis, the method of transmission by this fly in this region being purely mechanical and direct.

(2) In other Diptera also, T. gambiense starts on a development in a precisely similar manner, but without getting so far, or persisting so long, as in Glossina palpalis.

(3) The observations and experiments of Koch, Stuhlmann, and others, show that T. brucii goes through a developmental cycle in G. fusca.

(4) It is probable that T. gambiense has an invertebrate host in which it completes its life-cycle in regions where it is indigenous, and it is possible that the true host may be Glossina fusca.

(5) Considerations of phylogeny indicate that the life-cycles of different trypanosomes should not be expected to be in all cases of one invariable type.

(6) The encystation observed in T. grayi indicates that contaminative infection may occur as well as inoculative.

V. Record of Observations.

The batches of flies used in the experiments here recorded were infected by feeding them once on an infected animal, in all cases a monkey. Those flies which did not feed on the infected animal were destroyed. The flies that had fed were kept alive by being re-fed on a "clean" animal, in almost all cases a guinea-pig, but sometimes a monkey. At first I used to feed the tsetses once every two days, but latterly I found it better to give the flies the chance of feeding daily. The method of feeding in all cases was as follows: The flies were kept in boxes with mosquito-netting at the sides and the box was placed on the skin (previously shaved) of the experimental animal, usually on the belly, so that the flies could feed through the gauze. As a rule they fed readily; indeed, care was necessary in handling the boxes to avoid getting one's fingers bitten through the gauze. The boxes containing the flies were kept over water, as without moisture the flies died quickly. The monkeys used in my experiments were species of Cercopithecus, either pygerythrus or smithi, and in all cases were infected, directly or indirectly, with trypanosomes from human cerebro-spinal fluid. The following

is the record of the infected monkeys used by me, copied from the records of the Sleeping Sickness Commission, and kindly supplied to me by Gray, who entered them in the book.

"Monkey 404.

"June 7th, 1905.—Inject 5 c.c. of cerebro-spinal fluid from case of sleeping sickness, 'Sengoma.' Active trypanosomes are present in this fluid.

"July 16th.—Trypanosomes have not appeared in this animal's blood. Re-inject animal with a few drops of blood from Monkey 420. Trypanosomes are numerous in the blood of this latter animal (see Pl. VIII, figs. 22–25).

"Aug. 2nd.—Trypanosomes have appeared in this animal's blood to-day for the first time.

"Sept. 9th.—Trypanosomes have been regularly present in this animal's blood up to now. To-day the animal was sent to Nairobi at the request of the P. M. O. for the use of Dr. Ross, who is about to conduct some experiments on trypanosome transmission with the local tsetse-flies."

"Monkey 420.

"May 23rd, 1905.—Inject this monkey subcutaneously with 2 c.c. of cerebro-spinal fluid obtained from case of sleeping sickness, 'Vikitikeza.' Trypanosomes are numerous in this fluid.

"June 7th.—Trypanosomes have appeared in this animal's blood to-day for the first time. The parasites are scanty in numbers, and are long and thin for the most part.

"July 13th.—Trypanosomes are numerous in its blood.

"Sept. 18th.—The animal died to-day. Trypanosomes have been constantly present in its blood from the beginning. Post-mortem examination revealed nothing of interest."

"Monkey 478.

"Sept. 14th, 1905.—Inoculate this animal subcutaneously with 15 c.c. of cerebro-spinal fluid from a case of sleeping sickness, 'Sengoma.' Trypanosomes are numerous in the fluid.

"Sept. 26th.—Trypanosomes have appeared in this animal's blood to-day for the first time.

"Oct. 15th.—Trypanosomes numerous. Twelve flies fed on this animal to-day.

"Oct. 19th.—Trypanosomes numerous. Twenty flies fed to-day.

"Nov. 12th.—Trypanosomes numerous. Twenty flies fed to-day.

"Nov. 27th.—Died this morning. Trypanosomes numerous in the blood. One trypanosome seen in a smear of the brain. Glands and spleen somewhat enlarged. Stomach surface shows a few minute black ulcers and several nematode worms. Lungs, heart, etc., are normal."

"Monkey 507.

"Oct. 17th, 1905.—Inject the intestinal contents, with a few drops of normal citrate solution, of one Glossina palpalis subcutaneously into this monkey. This fly had fed on Monkey 478 some forty-six hours previously and was found on dissection to be swarming with trypanosomes (see p. 234).

"Dec. 5th.—This animal's blood has been regularly examined twice a week up to the present, but trypanosomes have never been found."

I begin the systematic record of my observations with the batch of July 31st, 1905. Previous to that date, however, I had made various tentative experiments and observations on the infection of the fly and on the changes of the trypanosomes in it, which I do not propose to record in detail.

Batch of July 31st, 1905.—The object of this experiment was to determine the changes undergone by T. gambi-

ense in G. palpalis during the earliest periods of infection. A number of tsetse-flies freshly caught near Entebbe were fed on Monkey 420. Smears were made at the same time of the monkey's blood. Four of the flies were dissected one hour after feeding, and four more five hours after feeding.

The monkey's blood examined fresh showed trypanosomes fairly abundant, some more slender, active, others stouter, less active. In smears fixed with osmic vapour, well-marked differentiation of slender, stout, and intermediate forms were seen (see P. R. S., B 78, Pl. XII, figs. 16–19). A count gave 45 slender (8 dividing), 48 intermediate (8 dividing), and 3 stout forms.

In the smears from the flies dissected after one hour similar types of trypanosomes were seen, but the differentiation into slender and stout forms appeared to be more pronounced, and fewer intermediate forms were seen (figs. 36–39). The impression given by comparison of the monkey's blood with smears made from the fly at this stage is that the intermediate forms of the former have become converted for the most part into the stouter type of the latter. A count gave 37 slender forms (6 dividing), 13 intermediate forms (2 dividing), and 18 stout forms.

Smears from the flies dissected five hours after feeding showed the differentiation still further advanced, especially of the slender forms, some of which showed the characteristic clear cytoplasm free from granules. Stout forms were also found (figs. 40–44). A count gave 21 slender forms (3 dividing), 6 intermediate forms (1 dividing), and 11 stout forms (1 dividing).

Aug. 1st (twenty-four hours after infection).—Four flies were dissected, but smears were only made of two. The smears, both of the red and black blood, showed sharp differentiation into clear slender and granular stout forms (see P. R. S., B 78, Pl. XII, figs. 1, 2, 10, and 11). A count gave 45 slender (7 dividing), and 13 stout forms (1 dividing).

Aug. 2nd (forty-eight hours after infection).—Three flies

were dissected and smears made of the blood. They showed trypanosomes of elongated, moderately stout form, and others of slender type. Dividing forms were also seen (P. R. S., B 78, Pl. XII, fig. 12).

A remarkable feature of this batch is the excess of slender forms over stout.

Batch of Sept. 8th, 1905.—A batch of flies, caught on the island of Kimmi, were fed on Monkey 420. Four flies were dissected after two hours, three after six hours, and three after twelve hours.

The smears made from the flies dissected after two hours showed slender, stout, and intermediate forms, with many division stages.

In the smears made from flies six hours after feeding, the differentiation of stout and slender forms was more marked. One fly contained Trypanosoma grayi as well as T. gambiense; unfortunately the preparations were a failure.

At twelve hours after feeding the differentiation of the stout and slender forms appeared to be complete.

Sept. 9th (twenty-four hours after infection).—Three flies were dissected and smears made from them. A fourth fly was dissected and the blood examined fresh. In the latter no trypanosomes were seen in the red blood; in the black blood they were found singly and in groups of two or three or even four. The single ones moved rapidly, with flagellum forwards. In the groups they were attached by the posterior ends with the flagella free; individuals thus attached were sometimes unequal in size. A group of three was observed in which four flagella could be distinctly seen, indicating division of one of them. A group of four was noted in which one was much smaller than the others, and one larger; this group separated into two couples; in each couple the trypanosomes were attached tête bêche, the two *nn* opposite and close together (fig. 110). The smears showed trypanosomes differentiated into the normal slender, clear, and stout, granular forms (P. R. S., B 78, Pl. XII, figs. 3–5 and 7–9). Dividing stages of both forms were common.

Sept. 10th (forty-eight hours after infection).—Three flies were dissected; the most successful preparation showed long, moderately stout or slender forms (see P. R. S., Pl. XII, fig. 15); the slender clear forms of the previous day wanting.

Sept. 12th (ninety-six hours after infection).—Three flies were dissected, and smears made of the blood. In the first two flies no trypanosomes were found; in smears of the third trypanosomes were found scantily in the red blood, but fairly abundant in the black. They were, for the most part, remarkable for their large size (p. 183, and Pl. X, figs. 97–101).

Batch of Oct. 1st, 1905.—This batch consisted of ten flies bred from pupæ. They were put on to Monkey 478, which was showing a fair number of T. gambiense in its blood, some smears being made from the monkey at the same time. Seven of the flies fed, three refused.

The smears of the monkey's blood showed trypanosomes chiefly of an indifferent type (figs. 1–5). None of the typical stumpy forms were found, but after some searching a few slender forms (fig. 5) were found. In all 83 indifferent forms, 10 slender, and 1 dividing form were counted in a smear.

Of the seven flies that fed one died before being examined; the remaining six were dissected on Oct. 2nd, 3rd, 4th, 5th, 6th, and 8th respectively. All their organs were examined very carefully, namely the pericardial fluid, Malpighian tubules, salivary glands, proventriculus, stomach, intestine, proctodæum, and in some cases the genitalia. In no case were trypanosomes found of any sort. Some of the flies were full of bacteria.

The negative result obtained in this batch was remarkable and difficult to explain. Two points call for notice—the very slight amount of differentiation in the trypanosomes of the monkey's blood, and the fact that the flies were bred in captivity and probably rather sickly and delicate; but whether the result is to be explained by either of these data cannot be asserted definitely.

Batch of Oct. 3rd.—This consisted of a few flies caught the day previously in the vicinity of Entebbe, and fed on

Monkey 478. The flies were kept in a large cage, with water and vegetation, in a situation where direct sunlight fell on the cage late in the afternoon. Smears of the monkey's blood made the day before (Oct. 2nd) showed a considerable amount of differentiation (figs. 6–10), in contrast with the smears made from the monkey the day previously (Oct. 1st). Stout, slender, and indifferent types could be distinguished; of the first 99, of the second 97, and of the third 34 were counted in one smear. Most of the stouter forms were of the typical stumpy form (figs. 9 and 10). Specially noteworthy is the length of the free flagellum in slender forms, and its shortness in the stout forms (compare figs. 6 and 7); by this character the indifferent forms could also be subdivided into two classes.

It is interesting that the trypanosomes from the blood of the same monkey on two consecutive days should show so little differentiation on one day, so much the next day.

Oct. 4th (twenty-four hours after infection).—Two flies were dissected. In the first a few trypanosomes of the stout type were seen in the black blood. A smear was made in which seven typical stout trypanosomes were counted. In the second fly no trypanosomes were found; the stomach contained a vast number of bacteria.

Oct. 5th (forty-eight hours after infection).—One fly was dissected, and various organs examined. Trypanosomes were found fairly abundantly in the red blood and the black blood. The sucking stomach contained a peculiar clot of blood, in which, however, no trypanosomes were found. The trypanosomes in the fresh condition appeared chiefly of the broad type, but one of more serpentine appearance with pointed posterior end was noted.

The smears made were unfortunately very unsatisfactory as regards preservation. In the best one, of the red blood, a few trypanosomes of moderately stout or moderately slender type (figs. 66–68) were found; in some parts of the smear they occurred in clumps, three or four together. In the smears of the black blood none were found.

Oct. 6th (seventy-two hours).—One fly was dissected; there was no blood in the digestive tract, only some brownish fluid in the intestine, in which two trypanosomes were seen. One smear was made, but no trypanosomes could be found in it.

Batch of Oct. 9th, 1905.—This batch consisted of two flies, both of which had been bred from pupæ in August, and had been kept since then in a fly-cage and fed regularly every two or three days on a fowl. The same fowl had been used for feeding the flies in the breeding-cage—that is to say, flies caught wild near Entebbe and kept in a large cage in order to obtain pupæ from them. The fowl became emaciated and of sickly appearance and died; its place was then taken by a second fowl. Unfortunately the fowl that died was not examined.

On Oct. 9th the two flies were fed on Monkey 409, which was showing a fair number of trypanosomes (T. gambiense) in its blood.

Oct. 10th (twenty-one hours after infection). — One of the flies were dissected. Both stomach and intestine were full of red and black blood respectively, and both were swarming with Trypanosoma grayi. In the proctodæum a few non-motile slender trypanosomes, apparently dead, were seen. No trypanosomes were found in the pericardial fluid, salivary glands, proventriculus, thoracic intestine, or sucking stomach. Examination of smears gave the following results: In all T. grayi was abundant, but prolonged searching was necessary to find T. gambiense, a specimen of which, from this fly, was figured by me (P. R. S., B 78, Pl. XII, fig. 14).

The smears of the red blood showed great variety of forms. The following types could be distinguished: (1) large forms, with hinder end narrow (figs. 218, 219); (2) large forms, with hinder end swollen, apparently about to divide (fig. 221); (3) quite small forms (fig. 220); (4) medium-sized, apparently representing stages in the growth of (3) into (1) and (2); (5) round forms, connected by transitions with (3) (figs. 222,

223); (6) slender forms (figs. 224–226); (7) dividing forms. A count gave the following numbers of each type:

	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Numbers .	10	34	7	5	19	26	2
Percentage .	9·7	33	6·79	4·85	18·44	25·24	1·94

The smears of the black blood showed the same types, (1) being perhaps rather more pronounced in its characteristics (figs. 226 *a*–231). A count gave the following results:

	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Numbers .	86	31	23	22	3	60	13
Percentage .	36·38	13	9·66	9·24	1·26	25·25	5·46

The two smears made from the proctodæum gave remarkable results. In one after much searching a single, slender form was found. In the other, vast swarms of trypanosomes were found in certain spots; in particular, one huge clump, quite visible to the naked eye, and resembling under a low power a section of spleen or other small-celled tissue. Away from these clumps trypanosomes were scanty. The majority of the trypanosomes were slender, Herpetomonas-like forms (fig. 232), but, unfortunately, badly preserved, having a macerated appearance, with *n* often broken up. A few stouter forms (fig. 233), and some pear-shaped forms without flagellum were also to be found. The fact that very scanty free trypanosomes were seen in the fresh condition indicates that the individuals found in the smears were probably all attached in clumps to the wall of the proctodæum, as noted in the fly of Nov. 14th (p. 245). The fact that the few free trypanosomes seen were non-motile, and that in the smear they appear macerated, indicates that the trypanosomes were moribund for some reason. (I have seen similar results follow from using a dissecting needle, which had been inadvertently dipped into borax carmine.)

Oct. 11th (forty-five hours after infection). The second fly of the batch was dissected; it was found to be very

anæmic, and its digestive tract contained no blood, but swarms of large bacteria. No trypanosomes were found.

Batch of Oct. 15th.—A number of flies, freshly caught near Entebbe, were fed upon Monkey 478.

Oct. 16th.—Four flies (one female, three males) were dissected, at about twenty-one, twenty-four, twenty-seven, and twenty-eight hours after infection respectively. In all of them trypanosomes were found both in the red and black blood, not very abundantly, but slightly more so in the black blood. They were for the most part of stout form, but some slender ones were seen also. In the black blood from the second fly two trypanosomes were seen adhering together by the hinder end; after a time a third added itself to them.

Smears were made from all the flies, both of red and black blood; they showed trypanosomes rather scanty in number, but sharply differentiated into slender and stout types (figs. 45–47); very few dividing forms were seen. In one smear, 33 stout and 4 slender were counted; in another, 88 stout, 4 slender, and 3 dividing.

Oct. 17th.—Five flies were dissected. The first fly (male, forty-five hours after infection) was plump and fairly full of blood, both red and black. Trypanosomes were seen fairly abundant in the red, still more so in the black blood; in the latter many of them were united by the posterior end into groups of as many as five. As these trypanosomes appeared active and healthy, as well as numerous, the experiment was made of injecting some of both red and black blood into a monkey (No. 509), but the injection was without effect, and the monkey never became infected (see p. 227).

Smears of the red blood showed trypanosomes sharply differentiated into stout and slender forms (figs. 76, 77), similar to those found the day previous. Smears of the black blood, however, showed a slightly different type, moderately stout, rather long, and characterised, for the most part, by considerable length of the free flagellum (figs. 73–75).

The second fly had the gut perfectly empty of blood, and contained no trypanosomes.

The third fly (female, forty-seven hours after infection) was gorged with blood. The trypanosomes were not very numerous; some were more slender, others stouter in form; in the red blood a slender one and a stout one were seen hanging on together.

Smears of the red blood showed stout forms, not very numerous, one of which was dividing (fig. 69). Smears of the black blood showed trypanosomes fairly numerous, varying from a moderately slender to a very stout type (figs. 70–72). In one smear were counted 17 slender, 25 stout, 21 intermediate, and 2 dividing forms.

The fourth fly (male, forty-eight hours after infection) was of emaciated appearance; the stomach was full of bacteria and contained no red blood; the intestine contained black blood in which were a few motile trypanosomes. Two smears were made, in each of which a single trypanosome of stout form was found.

The fifth fly (male, fifty hours after infection) had a small amount of red blood, in which no trypanosomes were seen, and a considerable amount of black blood, in which a few active trypanosomes were seen. In the smears, however, no trypanosomes could be found.

Oct. 18th.—Three flies dissected and examined. The first fly (male, sixty-nine hours after infection, not re-fed) showed a small amount of blood in the intestine, black posteriorly, reddish anteriorly. A few motile trypanosomes were seen; none in the proventriculus. In a smear a fair number of trypanosomes were seen, slender or moderately stout in type, some of them very granular; unfortunately the stain was very faint.

The second fly (male, seventy hours after infection, not re-fed) showed a similar condition to the last. A fair number of active trypanosomes were seen in the intestine, some of them long and moderately slender, others broader. Three trypanosomes, originally quite separate, were seen to come together and adhere by their posterior ends. No trypanosomes were found in the proventriculus, proctodæum, or

sucking stomach. The two smears made showed trypanosomes for the most part long and moderately stout (fig. 87), others more slender (fig. 86), and others again stouter (fig. 88), all of them very granular and very different in form and appearance from the two types found at twenty-four hours.

The third fly (female, seventy-two hours, re-fed at fifty-one hours after infection) had the digestive tract gorged with blood, red in the stomach, black in the intestine. No trypanosomes were found in the red blood, but in the black blood as many as three or four in a field (3 mm. apochr. immersion); at first very active, they soon became sluggish under observation. A stout one was seen with very distinct undulating membrane, and with *n* and *N* apparently dividing (fig. 109). Another rather smaller one had the undulating membrane distinct, and the cytoplasm containing refringent granules (fig. 107). A third was long and slender, with groups of refringent granules (fig. 108). Of the red blood five smears were made, and, after much searching, two trypanosomes were found in one of them; one of these was damaged, the other (fig. 82 *a*) was of extremely large size, very granular and vacuolated, and in process of division.

My two smears of the black blood show fairly numerous trypanosomes (figs. 83–85), most of them remarkable for their length and large size, varying in type from slender to stout; they are nearly always very granular and frequently vacuolated. Dividing forms were also seen.

Oct. 19th (ninety-four to ninety-eight hours after infection). —Three flies dissected, two male and one female; the two first had been re-fed Oct. 17th, the last re-fed Oct. 18th. All three flies appeared healthy and full of blood, red and black, but no trypanosomes were found in any of them. Numerous smears were made of each and carefully searched, but no trypanosomes could be found.

Oct. 20th (118–119 hours after infection).—The last two remaining flies (both males) were dissected and examined. No trypanosomes could be found in the digestive tract, neither in the fresh condition nor in smears.

Batch of Oct. 19th, 1905.—A number of freshly-caught Entebbe flies were fed on Monkey 478. At the same time smears were made of the monkey's blood, which showed trypanosomes rather scanty but comprising typical long, slender, and short, stumpy forms, as well as others of more indifferent type (figs. 11–13).

Oct. 20th (twenty-four hours after infection).—One fly dissected. The digestive tract was gorged with blood. Trypanosomes were very scanty in the red blood, more abundant in the black. Smears were made of both, and showed the typical slender and stout forms well differentiated, but rather scanty in number; the stout forms rather in excess.

Oct. 21st (forty-six to fifty hours after infection).—Four flies were dissected (three males, one female). In all of them only black blood was found; in one no trypanosomes were seen, but in the other three flies active trypanosomes of moderately slender type were noted. Smears made showed trypanosomes present, but scanty in number, in all four flies. The trypanosomes were long, varying from moderately slender to moderately stout in type, usually very granular, sometimes vacuolated (figs. 78–80). Dividing forms were seen.

Oct. 22nd.—Three flies were dissected. The first fly (female, seventy hours after infection; re-fed Oct. 21st) had the stomach and intestine very full of red and black blood respectively; the black blood contained a few crystals. Trypanosomes were fairly numerous in the black blood and of slender, active type, similar to T. grayi. One that was watched moved rapidly "en flêche" across the field, and was difficult to keep in view. None were seen in the red blood or in the proventriculus. Smears were made both of red and black blood; in the former no trypanosomes were found. In the smears of the black blood the trypanosomes were not very numerous, but very uniform in type (figs. 93–96). They were all of considerable length, moderately stout or moderately slender. (Their slender appearance in life is perhaps to be taken in a proportionate sense—that is to say, it was their

length which gave the impression of slenderness). Their cytoplasm was not granular or vacuolated, and they appeared perfectly healthy and normal; the free flagellum was relatively short; *n* relatively large, sometimes rod-shaped. No dividing forms were seen.

The second fly (male, seventy-one hours after infection, re-fed Oct. 21st) showed exactly the same condition as the last, and in this case also no trypanosomes were found in the red blood, but in the black blood they were numerous; some more slender in appearance and active in movement, progressing rapidly en flêche; others stouter, slower in movement. Smears were made of the black blood; unfortunately the preparations were not very successful, but they showed trypanosomes similar to those seen in the first fly (figs. 89–92), some very slender.

The third fly (female, seventy-two hours after infection, re-fed Oct. 21st) showed the same condition as the two already mentioned. In the black blood a few trypanosomes were seen, both slender and stout, but less active than those seen in the other two flies. The smears made were defective, but in one of them two trypanosomes of rather stout build were seen.

Oct. 23rd (ninety-four to ninety-eight hours after infection, re-fed Oct. 21st). The five remaining flies were dissected; in all of them both red and black blood was present in the digestive tract; the black blood contained numerous crystals. No trypanosomes were found in the red or black blood, nor in the proventriculus nor proctodæum. Smears were made of the blood, but prolonged searching failed to reveal any trypanosomes.

The remaining eleven flies of this batch were examined at intervals up to Nov. 1st, on which date the last seven were examined; in no case were any trypanosomes found.

Batch of Nov. 1st, 1905.—Some freshly-caught Entebbe flies were fed on Monkey 405, which, however, was showing very few trypanosomes in its blood.

Nov. 2nd.—Some of the flies were dissected; no trypanosomes were found in them with the exception of one, which

was found swarming with T. grayi. Five smears were made, but unfortunately no note was kept as to the parts of the gut from which the smears came—an oversight much to be regretted, as the preparations show trypanosomes of a remarkable type; slender ones varying from small (figs. 235, 236) and medium-sized (fig. 241) to a very great length (figs. 242–244). In some of the very long ones *n* could not be made out clearly, perhaps on account of defective preservation; in others *n* distinct. Large forms also occur (fig. 238), and a form with *N* posterior (fig. 234) is also common. In nearly every case *n* is well in front of *N*; only occasionally *n* at the side, and in two cases noted to be behind *N*. A few dividing forms were seen. In the preparations fixed with absolute alcohol the trypanosomes frequently appeared very granular, and full of chromidia.

Batch of Nov. 3rd, 1905.—A batch of Glossina palpalis, freshly caught, was fed on Monkey 478 (see p. 227), which was showing a fair number of trypanosomes in its blood. Smears were made at the same time of the blood of the monkey. The batch of flies was re-fed on healthy animals on the 5th, 7th, and 8th, and after that daily, until the flies had all been used up for dissection and examination.

The smears of the monkey's blood showed trypanosomes fairly abundant and of various types (see figs. 14–17). Of 212 trypanosomes counted, it is noteworthy that only one was dividing; the remainder comprised the following types of form: stumpy forms (figs. 16, 17), 82 (= 38·1 per cent.); long forms (fig. 14), 63 (= 31·2 per cent.); intermediate forms, slender, 28 (= 12·6 per cent.); intermediate, broad (fig. 15), 38 (= 17·5 per cent.). This shows a slight preponderance of stout over slender types, roughly 4 : 3.

Nov. 4th (twenty-four hours approximately after infection).—Three flies were dissected and examined, and smears made from each. In all of them active trypanosomes were found easily in the black blood, when examined fresh, but a hasty examination of the red blood did not reveal any trypanosomes;

they were found, however, in the smears of both red and black blood. The trypanosomes showed pronounced differentiation into slender and stout types (figs. 48–50), the latter greatly preponderating in number. A small number were seen to be dividing, in nearly all cases stout forms. Examination of seven slides gave the following results:

	Slender.	Stout.	Dividing.
1st fly, red blood	0	11	0
1st fly, red blood	15	64	1
1st fly, black blood	0	23	0
2nd fly, red blood	1	29	0
2nd fly, black blood	10	33	6
3rd fly, red blood	2	7	0
3rd fly, black blood	9	269	4
Total	37	436	11

This gives roughly twelve stout to one slender form.

Nov. 6th (seventy-two hours after infection).—One fly was dissected; the red blood examined fresh showed no trypanosomes, but in the black blood some active trypanosomes were found, chiefly of slender type; one large stout form, however, was seen. Three smears of the black blood were made, but put aside and fixed and stained in England; only on one of them were two trypanosomes found after much searching; both, unfortunately, were badly preserved.

Nov. 7th (ninety-six hours after infection).—Three flies were dissected and all their organs carefully examined for trypanosomes. The flies were apparently healthy and contained both red and black blood. In the first fly (male) a few scarce trypanosomes were noted (by my colleague F. M. Tulloch) in both the red and black blood, but in four smears made (two of each kind of blood) none could be found after prolonged searching. In the second fly (female) one active trypanosome was seen in the black blood, after much searching, but in the smear that was made none could be found. In the third fly (female) no trypanosomes were found.

Nov. 8th (120 hours after infection).—Five flies (four males, one female) were dissected and examined for trypanosomes. In each case the examination was extended to the red and black blood, the proventriculus and proctodæum, and either the testes and seminal vesicles, or the ovaries and the larva (present in one case). No trypanosomes were found.

Nov. 10th (168 hours after infection).—Three flies (all males) were dissected and all organs carefully examined. In one of them nothing was found; the other two were found to be infected with Trypanosoma grayi.

In the first fly the intestine contained a small amount of black blood, in which were corpuscles of normal appearance. No trypanosomes were found in the proventriculus, black blood, testes, or seminal vesicles, but the proctodæum contained slender, very active, and rapidly motile trypanosomes. One smear was made which showed fairly numerous trypanosomes of moderately slender type (figs. 132–135). Round forms (fig. 136) also occur, and others transitional (fig. 137), between the round forms and the prevailing slender type. No dividing forms were seen, nor any encysting forms (for further description see p. 195).

In the second fly also the intestine contained a fair amount of black blood and no red. Trypanosomes were found swarming in the proctodæum and black blood; none were found in the proventriculus, the testes, or the seminal vesicles. Smears were made of the proctodæum and the black blood. The smears of the proctodæum showed an immense number of slender forms with very long flagella, *n* large, transverse, always in front of *N* (figs. 144–146), also a few plumper, shorter forms, with the flagellum short and thick, as if being retracted, as in encysting forms, but without any cyst-wall (figs. 148–150); and, finally, a very few cysts, which, however, always showed the cyst-wall damaged and spread out, probably owing to the thinness of the smear; no dividing forms seen, with one doubtful exception (fig. 147). In the smears of the black blood trypanosomes were also found very abundantly and of types startlingly different from those in

the proctodæum. The trypanosomes exhibited great variation of form, size, and structure, but could be divided into four types: (1) The prevailing type was a large vermiform trypanosome with a very short, free flagellum, the anterior end blunt, and the posterior end narrow and drawn out (figs. 138–139); (2) others had the anterior end more tapering, the posterior more or less swollen (fig. 140); (3) a certain number were broad and fat, as if about to multiply by division (fig. 142); in addition to these three types there were found (4) many small or medium sized, as if resulting from recent division (fig. 141); on the other hand, division stages were extremely rare. In all these forms from the black blood the relative positions of *N* and *N* were extremely variable (for further details see p. 190). A count made from the two best preparations gave the following numbers for the various types:

	1. Vermiform.	2. Tadpole-like.	3. Large, fat.	4. Young, small.	5. Young, medium-sized.	6. Dividing.
1st preparation	80	20	14	65	34	0
2nd preparation	197	152		101	34	5
Total	277	186		166	68	5
Percentage	38·6	26·49		23·64	9·67	0·71

Nov. 11th (192 hours after infection).—One fly (male) was dissected; the intestine contained a small quantity of black blood; the stomach contained no blood but quantities of bacteria. No trypanosomes were found in the black blood, proctodæum, stomach, proventriculus, sucking stomach, testes, or seminal vesicles.

Nov. 12th (216 hours after infection).—Three flies (all males) were dissected; two of them contained red and black blood, one only black; in all three the stomach, intestine, proventriculus proctodæum, and genitalia were examined; no trypanosomes were found.

Nov. 13th (240 hours after infection).—Three flies were dissected. The first fly (male) was gorged with blood, both

red and black; no trypanosomes were found in the red or black blood, the proctodæum, salivary glands, or genitalia. The second fly (male) had the intestine empty except for a small quantity of blackish fluid in the intestine; the stomach was crammed with bacteria; no trypanosomes were found in the stomach, intestine, proctodæum, proventriculus, salivary glands, or genitalia.

The third fly (female) had a small quantity of red blood in the stomach, and the intestine full of black blood; it was found to be swarming with Trypanosoma grayi throughout the stomach, intestine, and proctodæum, but no trypanosomes were found in the salivary glands, larva, or proventriculus; one was found, however, in a teased-up ovary, but this was probably accidental; these excessively motile parasites are often let free during dissection by accidental ruptures of the gut-wall, and swarm out into the salt solution in which the dissection is performed.

Examination of the smears made from this fly gave the following results: In the red blood (figs. 151–155) trypanosomes were excessively abundant, for the most part of moderate size, fairly slender, the free flagellum often very short, sometimes long, and the relative positions of *N* and *n* extraordinarily variable (see p. 190). A count gave the following numbers of different types:

	Large, slender.	Large, stout.	Small.	Medium-sized.	Dividing.
Numbers .	240	12	157	41	9
Percentage .	52·28	2·6	34·2	8·93	1·96

In the black blood also the trypanosomes were very abundant and in the main similar in type to those in the red blood, perhaps rather more slender, more active in appearance (figs. 156–162); but in addition there occurred a small number of slender Herpetomonas-like forms, distinguished at once by their very slender form and transparent appearance; they stain feebly, and also have *n* in front of *N* (figs.

163, 164). A count gave the following numbers of the different types:

	Large, slender.	Large, stout.	Small.	Medium-sized.	Slender, H-like.	Dividing.
1st smear . .	248	90	27	27	103	9
2nd smear . .	258	24	37	13	94	8
Total . . .	506	114	64	40	197	17
Percentage .	53·94	12·15	6·82	4·26	21	1·81

Of the contents of the proctodæum I have two smears; in one, which I may call the pure smear, there are no blood-corpuscles; in the other there is a slight admixture of black blood and the preparation contains blood-corpuscles. The trypanosomes show the same types as in the black blood (figs. 165–170), and a count of the pure smear gave the following results:

	Large, slender.	Large, stout.	Small.	Medium-sized.	Slender, H-like.	Dividing.
Numbers . .	258	62	21	4	106	24
Percentage . .	54·31	13	4·42	0·84	22·31	5·05

Comparison of the figures shows that the percentage of Herpetomonas-like forms is about the same in the black blood and the proctodæum.

Nov. 14th (264 hours after infection).—Three flies (all males) were dissected. In two of them the stomach contained no red blood, but numerous bacteria, and the intestine contained blackish fluid; in both of these the proventriculus, stomach, intestine, protodæum, testes, and seminal vesicles, and in one the salivary glands, were examined without finding any trypanosomes.

In the third fly the digestive tract was found gorged with blood, both red in the stomach and black in the intestine. Trypanosoma grayi was found present in stupendous numbers throughout the stomach, intestine, and proctodæum; in the last named the trypanosomes were found both

free and swimming actively and also adherent to the wall in large patches, resembling, under the low power of the microscope, patches of mould, the whole mass quivering and vibrating with the movements of the flagella. No trypanosomes were found in the testes, seminal vesicles, salivary glands, or proventriculus.

Examination of smears gave the following results: The red blood showed a preponderance of large forms (fig. 173), together with a certain number of small or medium-sized trypanosomes (fig. 171) and a few division stages; slender forms (fig. 172) were also found, but were very scarce. A count gave the following results.

	Large.	Medium.	Small.	Male.	Dividing.
Numbers .	237	64	63	3	14
Percentage .	62·2	16·79	16·5	0·78	3·6

The transitional region between the red and black blood showed a similar state of things, but the trypanosomes were rather smaller, not attaining such great length as in the red blood (figs. 174–176). Division stages were frequent, and a series has been drawn from the two smears of this region (figs. 204–216). No slender forms were found.

The black blood (figs. 177–182) showed in the smears a few large forms, and some of medium size (fig. 177), but the great majority were small forms, like those recently originated from division. In one slide forms were found, which, by their shape—especially the pointed hinder extremity (fig. 178)—approached the Herpetomonas type, but true H-forms were not found. A few round forms also were found. A count resulted as follows:

	Large or medium.	Small.	Round.
Numbers	162	749	23
Percentage	17·34	80·1	2·46

Thus over 80 per cent. were small forms, all of them with *n* in front of *N*, or at the side; none with *n* behind *N*. In

the larger forms the relative position of *n* and *N* varies greatly (see p. 190). Dividing stages were very rare; one is figured (fig. 180).

The smears[1] from the proctodæum showed free forms and all stages of encystment. The free forms show two types: (1) larger, broader forms, resembling the small forms seen in the black blood (figs. 183, 186); (2) slender, Herpetomonas-like forms with narrow bodies pointed posteriorly, and very long flagella, faintly stained (figs. 184, 185, 187, 188). The encysting forms, though forming a continuous series, may be divided into (3) forms with flagellum recognisable (fig. 189–193); (4) pear-shaped with flagellum retracted (figs. 194–198); (5) ripe cysts, more or less spherical (figs. 199–202). A count gave the following results:

	1. Ordinary.	2. H-like.	3. Encysting flagellum present.	4. Flagellum retracted.	5. Ripe cysts.	6. Round forms.	7. Dividing forms.
Number .	41[2]	60	38	62	30	2	1
Percentage.	17·48	25·58	16·22	26·42	12·82	·84	·42

Nov. 15th (288 hours after infection).—Two flies, the last survivors of the batch, were dissected, one a male, the other a female, containing a larva. Both were gorged with blood, red in the stomach, black in the intestine, of both the proventriculus, stomach, intestine, proctodæum, genitalia, and in the female the larva were examined, but no trypanosomes were found.

Stomoxys Experiments.

Nov. 17th.—A batch of Stomoxys sp. fed on Monkey 510, which was showing a fair number of trypanosomes.

Nov. 18th.—Four flies dissected and examined; in all try-

[1] Although the proctodæum contained no blood, my smears show a few blood-corpuscles. I think these must have come from the serum used in making the preparations.

[2] One, and only one, of this number had *n* behind *N*.

panosomes were found in the fresh state. Smears were made and put by for examination. In the smears of two flies no trypanosomes were found; in a third fly they were found in the smears of red blood, not in the black; in the fourth fly they were found both in the red and the black blood. The trypanosomes were slender and stout forms perfectly typical in character (figs. 51–55), the former scarce, and some of the latter dividing. In a smear from one fly 66 stout (6 dividing) were counted, but no slender; in a smear from the other fly 28 stout, of which 11 were dividing, and 4 slender were counted. The high proportion of division-stages is remarkable.

Nov. 19th.—The remaining Stomoxys, 20 in all, were dissected and examined, but no trypanosomes were found in any of them.

Mansonia Experiments.

Nov. 19th.—Two mosquitoes (Mansonia sp.) were fed on Monkey 478.

Nov. 20th.—One was dissected and somewhat hastily examined without any trypanosomes being noted. In the smears, however, trypanosomes were found abundantly, of pronounced slender and stout type (figs. 56–60). In one smear 120 stout and 60 slender were counted; in another, 78 stout, 34 slender; no dividing forms were seen.

Nov. 20th.—One Mansonia was dissected, but no trypanosomes were found, and unfortunately no smears were made.

Tæniorhynchus Experiments.

First batch, Nov. 19th.—Four mosquitoes (Tæniorhynchus sp.) fed on Monkey 478.

Nov. 20th.—Two dissected; in each active trypanosomes were noted. In the smears numerous typical slender and stout forms were found (figs. 61–65). Very few were dividing. In one smear were counted 78 stout, 41 slender forms (of which 4 were dividing); in another smear, 89 stout, 45 slender (1 dividing).

Nov. 21st.—Two dissected; a few rare active trypano-

somes found, others motionless, apparently dead. In the smears made a fair number of trypanosomes were found, mostly of stouter form, often very vacuolated, and with *N* broken up (figs. 81–82); one slender form, apparently much macerated, was found. No dividing forms were seen.

Second batch, Nov. 20th.—A number of Tæniorhynchus fed on Monkey 478. Some were dissected after forty-eight hours (Nov. 22nd); in one a motile trypanosome was seen, but in the smear made from this none were found. The four remaining were dissected after seventy-two hours (Nov. 23rd); the blood had become reduced to a mass of dancing granules, amongst which blood-corpuscles were absent or very rare. In three of the mosquitoes no trypanosomes were found; in the fourth some active trypanosomes of stout type were found, but in the smear made none were found.

The experiments with Stomoxys and mosquitoes were highly incomplete, and it is to be regretted that more were not carried out; but so far as they go they show one interesting result, namely that the trypanosomes become differentiated into exactly the same two forms, stout and slender, as they do in Glossina. Further, the trypanosomes in the digestive tract one day after feeding appeared to have multiplied, as judged both by their number and by the frequent occurrence of dividing forms.

Examination of Lice from Sleeping Sickness Patients.

The Father Superior of the Sleeping Sickness Refuge, conducted by the Algerian White Fathers at Kisubi, kindly caused lice to be collected for me from patients in three degrees of the disease—early, medium, and late. I dissected ten lice of each batch and examined all their organs very carefully. The gut contained in all cases numerous cocci of fair size, in couples or clusters; the hinder part of the intestine frequently contained very large, active, rod-shaped bacilli. No trypanosomes were found in any case.

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APPENDIX.

Since the above was written a detailed memoir[2] on the cytology of trypanosomes has been published by Salvin-Moore and Breinl, in which the authors take a very different view of the nature of the nuclear apparatus from that taken by me. They regard the kinetonucleus as an "extra-nuclear centrosome" or blepharoplast, comparable with an intra-nuclear centrosome or karyosome contained within the principal nucleus. The extra-nuclear centrosome is described as "a granule or small group of granules which stain like the intra-nuclear centrosome," and the flagellum is stated to arise from one or more of these granules. In development the extra-nuclear centrosome is stated to arise from the intra-nuclear centrosome, which divides into two halves within the nucleus, after which one half is extruded from the nucleus to form the extra-nuclear centrosome.

[1] This title is somewhat misleading; the author states (p. 83), "I have never seen any sign of an intra-corpuscular stage of the trypanosoma."

[2] Salvin-Moore, J. E., and Breinl, A., "The Cytology of the Trypanosomes," Part 1, 'Ann. Trop. Med. Parasitology,' vol. i, pp. 441–480, Pls. xxxviii–xlii.

I am unable to agree with Salvin-Moore and Breinl in regarding the kinetonucleus as of centrosomic nature. On the contrary, I agree with the recently expressed view of Keysselitz, to the effect that "Der Blepharoplast stammt vom Kern ab und besitzt Kernnatur" ('Arch. Protistenkunde,' x,

Text-fig. B.—Trypanoplasma sp. from a pike (Esox lucius), Sutton Broad, Norfolk, to show the kinetonucleus, larger and more darkly stained than the trophonucleus, and the two blepharoplasts, from each of which a flagellum arises; the cytoplasm contains numerouse oarse staining granules. Osmic vapour, Giemsa, × 2000 linear.

p. 129); I should differ from Keysselitz in the use of the word blepharoplast, which, in my opinion, should be used for bodies of centrosomic and not of nuclear nature. I am of opinion, further, that Salvin-Moore and Breinl have confused two distinct structures under the name "extra-nuclear centrosome"; namely, the kinetonucleus, a chromatic body, and the true blepharoplast, a centrosomic or achromatic body. It

is unfortunate from this point of view that these investigators have studied only pathogenic trypanosomes (T. gambiense, brucii, and equinum), in which the kinetonucleus happens to be extremely minute and does not contrast sharply with a blepharoplast; a peculiarity which may be characterised as a "morphological curiosity." If they had studied forms with a large kinetonucleus, like T. grayi, I do not think they would have fallen into this error. Still more decisive against the view of Salvin-Moore and Breinl, it seems to me, is the genus Trypanoplasma (text-fig. B), in which *n* is as large as or even larger than *N*, and in which two distinct blepharoplasts, from each of which a flagellum arises, can be made out plainly in good preparations; pace Salvin-Moore and Breinl, who would not concede, probably, that good preparations can be made anywhere but in Liverpool. Finally, I am unable to agree that a structure of the same nature and reactions as the kinetonucleus exists in the interior of the trophonucleus. Here, again, a form such as T. grayi is very instructive; the large kinetonucleus of this form stains very intensely and of a different tint from the granules composing the nucleus. If a structure similar to the kinetonucleus were contained in the nucleus it would be impossible to overlook it. After what I have said above it is not necessary to point out that for Trypanoplasma the notion that the trophonucleus contains a body similar to the kinetonucleus becomes an absurdity.

The difference, however, between the views of Salvin-Moore and Breinl and of myself is largely one of terminology, since the Liverpool investigators admit that the kinetonucleus arises from the nucleus. They describe it as originating by division of a karyosome, which they compare to the karyosome described by Schaudinn, in Coccidium schubergi; a karyosome of this kind is a chromatic body, even if it contain a centrosome. Further, Salvin-Moore and Breinl regard the two nuclei (*n* and *N*) of a trypanosome as comparable to two differentiated gamete-nuclei, a comparison which I consider far-fetched and misleading in the highest degree, but

which at least shows that they regard both *n* and *N* as bodies of the nature of true nuclei. I hold that the terms centrosome and blepharoplast should not be applied to bodies which are recognised to be of the nature of nuclei; I differ, therefore, from the Liverpool investigators, as from Keysselitz, mainly in a matter of the use of words.

The memoir of Salvin-Moore and Breinl confirms me in the view I have expressed above; namely, that the body of a trypanosome contains two distinct nuclei, and that each of these two nuclei has a centrosomic corpuscle in connection with it; for that, in connection with the trophonucleus, I use the term centrosome simply, since its function is mainly related to division of the nucleus; for that in connection with the kinetonucleus I use the term blepharoplast, since the flagellum takes origin from it. I can imagine that this type of structure may be susceptible of variations and additions; the centrosome might be imbedded in a chromatic mass or true karyosome; the blepharoplast might be lodged in the centre of the kinetonucleus; in either case the essential nature of blepharoplast and centrosome would not be affected.

With reference to my diagram given above (Text-fig. A, p. 174), I should explain that I have purposely given a negative picture, so to speak, of the nucleus and centrosome; that is to say, I have represented the centrosome as a distinct black granule in the midst of colourless chromatic granules making up the trophonucleus; had I represented the centrosome as it really is, namely, as a colourless granule in the midst of deeply staining chromatin-granules, it would have been as difficult to see in my drawing as it usually is in the actual preparations of trypanosomes.

Salvin-Moore and Breinl deny any differentiation of trypanosomes in the blood; they state that the three types, slender, intermediate, and stout, distinguished by me, are "arbitrarily chosen examples in a continuous series of dimensions." To this I reply, first, that it has never been disputed that the different types are connected by transitions, since both the slender and stout forms are differentiations, more or

less strongly marked, of the common intermediate type; secondly, that the difference between the extreme forms is not one of dimensions alone, but of points of structure and morphology which Salvin-Moore and Breinl have overlooked. I am content to let my figures speak for themselves.

Since my work relates almost exclusively to the development of Trypanosoma gambiense in the tsetse-fly, I have no comment to offer on the cycle in the blood, with formation of latent forms, described by Salvin-Moore and Breinl; a discovery of the highest importance, if true, but which does not, in my opinion, disprove the existence of a developmental cycle in an invertebrate host. It is possible, however, that the pathogenic trypanosomes as a group may owe their peculiar properties to having become adapted exclusively to vertebrate hosts; but in that case the problem of transmission and infection becomes difficult to understand. Salvin-Moore and Breinl state (p. 446) that the blood during negative phases, "even if it be properly filtered, is still capable of infecting." This is a very important statement, and I look forward with much interest to the publication by the authors of the evidence on which it is based. At present they have given us none.

Lister Institute, January 15th, 1908.

DESCRIPTION OF PLATES 8—13.

Illustrating Prof. E. A. Minchin's paper on "Investigations on the Development of Trypanosomes in Tsetse-Flies and other Diptera."

All the figures are drawn with the camera lucida to a magnification of 2000 diameters, except Figs. 102–110, Figs. 122–125, and Figs. 127–130 on Pl. 10, which are freehand sketches.

PLATE 8.

Trypanosoma gambiense.

Figs. 1–5.—From the blood of Monkey 478, on October 1st, 1905 (pp. 176 and 230).

FIGS. 6–10.—From the blood of Monkey 478, on October 2nd, 1905 (pp. 176 and 231).

FIGS. 11–13.—From the blood of Monkey 478, on October 19th, 1905 (pp. 176 and 237).

FIGS. 14–17.—From the blood of Monkey 478, on November 3rd, 1905 (p. 239).

FIGS. 18–21.—From the blood of "fresh-fly monkey" (p. 175).

FIGS. 22–25.—From the blood of Monkey 420, on July 16th, 1905 (p. 226).

FIGS. 26–29.—From the blood of male Chimpanzee, eighteen days after inoculation with cerebro-spinal fluid.

FIGS. 30–35.—From human cerebro-spinal fluid.

FIGS 36–39.—From the stomach of Glossina palpalis one hour after feeding on an infected monkey, on July 31st, 1905 (p. 228).

FIGS. 40–44.—From the stomach of G. palpalis five hours after infection on July 31st, 1905 (p. 228).

FIGS. 45–47.—From G. palpalis twenty-four hours after infection, on October 16th, 1905 (p. 234); Fig. 46 from the red blood; Figs. 45 and 47 from the black blood.

FIGS. 48–50.—From G. palpalis twenty-four hours after infection, on November 4th, 1905 (p. 240); red blood from second fly.

FIGS. 51–55.—From Stomoxys sp. twenty-four hours after infection, on November 18th, 1905 (p. 247); Figs. 51 and 52 from one fly; Figs. 53–55 from another.

FIGS. 56–60.—From Mansonia sp. twenty-four hours after infection, on November 20th, 1905 (p. 247).

FIGS. 61–65.—From Tæniorhynchus sp. twenty-four hours after infection, on November 20th, 1905 (p. 247).

PLATE 9.

Trypanosoma gambiense.

FIGS. 66–68.—From Glossina palpalis forty-eight hours after infection, on October 5th, 1905 (p. 231); red blood.

FIGS. 69–72.—From G. palpalis forty-eight hours after infection, on October 17th, 1905, third fly (p. 235); Fig. 60, red blood; Figs. 70–72, black blood.

FIGS. 73–77.—From G. palpalis forty-eight hours after infection, on October 17th, 1905, first fly (p. 234); Figs. 73–75, black blood; Figs. 76, 77, red blood.

FIGS. 78–80.—From G. palpalis forty-eight hours after infection, on October 21st, 1905, first, second, and fourth flies respectively (p. 237).

Figs. 81, 82.—From Tæniorhynchus sp. forty-eight hours after infection, on November 21st, 1905 (p. 248).

Figs. 82 *a*–85.—From Glossina palpalis seventy-two hours after infection, on October 18th, 1905, third fly (p. 236); Fig. 82 *a*, red blood; Figs. 83–85, black blood.

Figs. 86–88.—From G. palpalis seventy hours after infection, on October 18th, 1905, 2nd fly (p. 236).

Figs. 89–92.—From G. palpalis seventy-one hours after infection, on October 22nd, 1905, second fly (p. 238).

PLATE 10.

Trypanosoma gambiense; other parasites of Glossina palpalis.

Figs. 93–96.—From G. palpalis seventy hours after infection, on October 22nd, 1905, first fly (p. 237).

Figs. 97–101.—From G. palpalis ninety-six hours after infection, on September 12th, 1905, third fly (p. 230); Figs. 97, 98, black blood; Figs. 99–101, red blood.

Figs. 102, 103.—Impressions of the two types of trypanosomes seen living in the stomach of G. palpalis twenty-four hours after infection with T. gambiense; sketched July 18th, 1905. (Zeiss. compens. oc. 12, apochr. imm. 3 mm., 1·40 Ap.)

Figs. 104–106.—Impressions of the forms of T. gambiense seen living in G. palpalis forty-eight hours after infection; sketched July 19th, 1905. (Lenses as in last.)

Figs. 107–109.—Impressions of the forms seen living in G. palpalis seventy-two hours after infection, on October 18th, 1905, third fly (p. 236).

Fig. 110.—Sketch to show how a couple of living trypanosomes were attached to one another, on September 9th, 1905 (p. 229).

Figs. 111–125.—Bacteria from the stomach of Glossina palpalis (p. 169); Figs. 111–121, from a preserved smear, × 2000; Figs. 122–125, sketched living.

Figs. 126–131.—Alga-like bodies from the stomach of the fly (p. 170); Fig. 126, from a smear, × 2000; Figs. 127–131, sketches drawn from the living bodies. Between Figs. 127 and 128 the outline of a monkey's blood-corpuscle is sketched for comparison of size.

PLATE 11.

Trypanosoma grayi from Glossina palpalis.

Figs. 132–137.—From November 10th, 1905, first fly, proctodæum (p. 241).

FIGS. 138–143.—From November 10th, 1905, second fly, black blood (p. 242).

FIGS. 144–150.—From November 10th, 1905, second fly, proctodæum (p. 241).

FIGS. 151–155.—From November 13th, 1905, third fly, red blood (p. 243).

FIGS. 156–164.—From November 13th, 1905, third fly, black blood (p. 243).

FIGS. 165–170.—From November 13th, 1905, third fly, proctodæum (p. 244); all drawn from the "pure" smear.

FIG. 171–173.—From November 14th, 1905, third fly, red blood (p. 245).

FIGS. 174–176.—From November 14th, 1905, third fly, transition from red to black blood (p. 245).

FIGS. 177–182.—From November 14th, 1905, third fly, black blood (p. 245).

FIGS. 183–185.—From November 14th, 1905, third fly, proctodæum (p. 246).

PLATE 12.

Trypanosoma grayi. Stages of encystation and division (except Fig. 217).

FIGS. 186–203.—From November 14th, 1905, third fly, proctodæum (p. 246).

Fig. 186.—Ordinary young form.

Figs. 187, 188.—Herpetomonas-like forms, free, not begun to form cysts.

Figs. 189–193.—Forms in which the formation of the cyst and the retractation of the flagelum is proceeding.

Figs. 194–198.—Forms in which the retractation of the flagellum and the formation of the cyst is more advanced. Figs. 194 and 195 show the condition with a flagellum vacuole.

Figs. 199–202.—Ripe cysts. Fig. 202 is from a thin smear and is damaged; the others are from a thick smear. In Figs. 200 and 201, *n* cannot be identified with certainty.

Fig. 203.—Division proceeding within a cyst, which is broken in making the smear.

FIGS. 204–216.—From November 14th, 1905, third fly, transitional region between red and black blood (p. 245).

Figs. 204, 205.—Earliest stage of division, the flagellum split at the base.

Figs. 206–208.—The splitting of the flagellum has gone further; in Fig. 207 *n* has divided; in Fig. 208 both *n* and *N* have divided.

Figs. 209, 210.—Splitting of flagellum complete, the two halves of *N* still connected by a band or fibre.

Figs. 211, 212.—Division of the body.

Fig. 213.—Flagellum and *n* divided, *N* still undivided.

Fig. 214.—Flagella distinct, *n* double, *N* single.

Figs. 215, 216.—Flagellum divided, *n* and *N* single.

Fig. 217.—Nearly complete division of a slender form of T. gambiense from stomach of G. palpalis two hours after infection.

PLATE 13.

Trypanosoma grayi from Glossina palpalis.

Figs. 218–226.—From October 10th, 1905, red blood (p. 232).

Figs. 226 *a*–231.—From October 10th, 1905, black blood (p. 233).

Figs. 232, 233.—From October 10th, 1905, proctodæum (p. 233).

Figs. 234–244.—From November 2nd, 1905 (p. 239).

The Nematocysts of Turbellaria.

By

C. H. Martin, B.A.,
Magdalen College, Oxford.

With Plate 14.

Prior to 1903 it was accepted by most zoologists that nematocysts were formed in three separate phyla of the animal kingdom—in Cœlenterates, Turbellaria and Mollusca.

If it were really the case that the nematocysts which undoubtedly occur in these three separate phyla are produced by the tissues of the creatures containing them, it would be an example of convergence without parallel in the animal kingdom.

For both in Æolids and Turbellaria there are instances of individual species containing nematocysts of two or three different kinds which are the exact duplicate of similar structures in the Cœlenterates.

In all other cases of convergence, though there may be a strong superficial resemblance in structures occurring in animals widely separate from one another, careful analysis shows that the resemblance never amounts to identity, as it does in this case.

As long ago as 1858 Strethill Wright explained the presence of nematocysts in Æolids on the hypothesis that they are derived from the Hydroids on which they feed, and in 1903 Grosvenor (**8**), after a searching investigation, raised this hypothesis to the level of an established fact.

Since it has been proved that the nematocysts of Æolids

are extraneous structures it seemed to me that the same would probably prove to be the case in Turbellaria, and the following paper is an account of the investigation which I have made on the subject.

The research was commenced in Scotland on Loch Tay and Loch Lomond, when I was working on the Scotch Lake Survey under Sir John Murray, to whom, for his kindness, I am greatly indebted.

In November, 1906, I had the opportunity of occupying the Oxford Table at the Naples Aquarium, and there I was enabled to examine some of the Marine Turbellaria, in which the presence of nematocysts has been suspected.

I should like to take this opportunity of thanking the staff of the Aquarium, and Professor Dohrn in particular, for the great kindness they showed me.

In April I had an opportunity of showing some preparations to Professor von Graff, and discussing the question with him.

Finally, I should also like to thank Professor Bourne for his great help in the preparation of the paper.

The nematocysts of Turbellaria were probably first observed by Oersted (**13**) in Microstoma lineare but he failed to recognise their true character, and described them as "Krugformige Drusen." They were subsequently examined by von Siebold (**17**) in 1848, and he leaves no doubt as to his recognition of their nature describing them as thread cells which "denen der Hydra auf ein Haar gleichen sollten."

Hallez (**9**) described the thread of the nematocysts in Microstoma lineare as "liquide qui se coagule légèrement au contact de l'eau," and put forward in common with M. Müller and Leuckart, the view that rhabdites were degenerate nematocysts. The essential difference between these two structures lies, of course, in the fact that whilst the rhabdite is a solid rod of more or less homogeneous nature, the nematocyst is a capsule containing a spirally rolled thread which can be everted under suitable conditions.

Von Graff (**5**), in his great work on Turbellaria in 1882, stated that the nematocysts of Microstoma can be distinguished from those of Hydra (1) by their smaller size, the length of the large capsule measuring 0·015 mm., and the thread 0·26—0·13 mm.; (2) by the presence of only four barbs. He contradicts Hallez, who had correctly described the nematocysts as lying in vacuoles, asserting that each lies in a single cell which (although on this point he seems to have been somewhat doubtful) possesses a cnidocil. He also described so-called intermediate forms between rhabdites and nematocysts in Polycystis (Macrorhyncus), Mamertinus, etc., and concluded that nematocysts were homologous with rhabdites ("so scheint die auch von Hallez acceptierte Homologie zwischen den Rhabditen und Nematocysten der Turbellarien ziemlich sicher begrundet, und wir konnen mit M. Müller und Leuckart die ersteren als niedere Zustande von Nesselorgane beobachten").

Fuhrmann (**4**) has more recently studied the large oval nematocyst in Microstoma. He finds that they measure ·0084—·0187 mm. in length, and that the distal end is closed by a lid, which springs off when the nematocysts explode.

The thread, according to his account, is solid, measuring about ·137 mm., and there are four long and four short barbs. Under these circumstances it would seem very difficult to understand how, with a solid thread, the explosion is effected, since his drawings show that the barbed base of the thread is everted.

In his latest work on Turbellaria in Bronn's 'Thierreich,' von Graff (**7**) places all the rhabdite-like structures—rhabdoids, pseudorhabdites, sagittocysts, and nematocysts—under a common category of Hyaloids.

(*a*) The rhabdoids measure from 0·16 μ to 87 μ, and are sub-divided into rhamnites (bent rods) and rhabdites, which are more resistent, cylindrical, or elliptical bodies. They may be formed either in the mesenchyme or in the epithelial cells.

(*b*) Pseudorhabdites are usually confined to the Alloiocœla, and are characterised by their granular appearance.

(*c*) Sagittocysts are confined to the Acœla, and consist of a membrane surrounding a cavity in which a solid needle-shaped body lies.

They seem to occur only in the genital regions at the period at which the male sexual products are mature, and are regarded as "Reizmittelm bei der Begattung."

(*d*) Nematocysts.

Von Graff summarises his views as to the connection between these various structures in the following passage:

"Von den als Ausganspunkt erscheinendem Pseudo-rhabditen, welche sich weder gegen das ungeformte Secret der Hautdrüsen einer noch gegen die strukturlosen Rhabditen andererseits scharf abgrenzen lassen, führt auf diese Weise, durch solche Rhabdoiden bei welchen sich ein hyalinen Mantel von der Kornigen Zentralmasse scheidet (Rhammiten) die schrittweise Differenzierung zu Sagittocysten und den verschiedenen formen von Nematocysten—Eine Anschauung die ich schon 1874 ausgesprochen habe."

The following list contains, I believe, the names of all the Turbellaria which have been described as possessing nematocysts. These which I have been able to examine are marked with an asterisk:

RHABDOCŒLA.

Section 1.—HYSTEROPHORA.

Fam. CATENULIDÆ.

Microstoma lineare.*
„ giganteum.*
„ rubromaculatum.*
„ papillosum.*
Stenostoma sieboldii.*

Section 2.—LECITHOPHORA.

Sub-section: KALYPTORHYNCHIA.

Fam. POLYCYSTIDIDÆ.

Polycystis naegelii.*
„ mamertinus.*

POLYCLADA.

Hyporhyncus armatus.
Anonymus virilis.
Stylochoplana tarda.

ALLOIOCŒLA.

Allostoma monotrochum.
Plessisia setosa.

Of all the Turbellaria, as far as I can discover—Microstoma lineare, Stenostoma sieboldii, Plessissia setosa, and Stylochoplana tarda are the only forms in which the actual expulsion of a thread from the nematocyst has been described.

In most of the other species of Rhabdocœls the so-called nematocysts are bodies of very small size in which the appearance of a coiled thread has been suspected.

The two Polyclads in which nematocysts have been described are unfortunately very rare Stylochoplana tarda and Anonymus virilis having only once been found.

Microstoma lineare.—In Microstoma lineare nematocysts are by means of universal occurrence. Du Plessis (**16**) described a variety from the deeper water of Lake Geneva in which nematocysts were absent. This form has more recently been raised to specific rank by Zacharias (**20**) under the name of Microstoma inermis.

I have twice found a similar form in Loch Tay at a depth of about 200 feet. It is characterised by the absence of eyes and nematocysts, but as nearly all littoral Rhabdocœls lose their eyes in deep water, and as I hope to show that the absence of nematocysts is merely the result of the absence of Hydra at this depth, I do not think the species can be maintained.

In those animals in which they occur nematocysts vary enormously in number, and it is fairly evident that they are most numerous in the forms from Scotch lochs in those individuals caught on stones on which Hydra rubra is very abundant.

In the living Microstoma the nematocysts may be seen to lie in vacuoles just under the skin. They may lie singly or to the number of three or four together; very frequently the same vacuole encloses nematocysts of different types. I have seen cases in which a vacuole enclosed one large barbed nematocyst with three small oval nematocysts, and in other cases I have seen the barbed form accompanied by the cylindrical form.

In Microstoma lineare from Loch Tay and Loch Lomond I have found three kinds of Nematocysts, which cannot be distinguished from the nematocysts of Hydra rubra occurring in the same regions. (*a*) The large barbed nematocysts measure, in an unexploded condition in balsam preparations, 12—15 μ length, 7—9 μ breadth, and when the nematocyst is discharged the length of the thread is about 400 μ. There are only three large barbs directed backwards at the end of the thickest portion of the thread. (*b*) The small cylindrical nematocysts measure in length 12 μ, in breadth 4 μ, and the length of the thread is about 300 μ. (*c*) There are also small spherical nematocysts with a thick thread and no barbs. In sections through a Microstoma nematocysts may be found lying in four different positions—(1) in the lumen of the gut, (2) in the cells of the gut, (3) surrounded by cells in the body cavity between the gut wall and the ectoderm, (4) in the ectoderm.

(1) In the gut of a Microstoma which has recently eaten a Hydra, the nematocysts may still be found lying in the tissue of the Hydra in the lumen of the gut.

(2) As digestion proceeds, the nematocysts are engulfed by the cells of the gut, and may be found lying to the number of two or three in the same cell.

(3) At a later period nematocysts can be found lying just outside the gut, sometimes free, but usually surrounded by three or four small cells. These cells seem to be mesenchymatous phagocytes, though I am not quite sure whether it is not possible for the cells of the gut itself to become free and

take up a wandering existence in the body cavity. Finally the nematocyst is transported to a position directly under the ectoderm; here it lies in the vacuole (vide. figs. 2—4) (which is not an artifact, since it can be seen in the living animal) surrounded by about six cells. The wall of the vacuole after a time becomes thinner and denser. There is one point of great interest as regards the orientation of the nematocysts under the skin. The large barbed nematocysts in their final position, always lie so that the thread, when it is discharged, will pass out of the animal, although they may lie pointing in any direction while they are still in the gut cells or the body cavity. This rule does not seem to hold good in the small cylindrical nematocysts, which, as far as I can see usually lie almost parallel to the surface. It is very difficult to say how such an orientation can be effected, but something of the same kind has been detected in Æolids, and I believe that the same difficulty is present in the nematocysts of the tentacles in Cœlenterates. In Hydra the tentacles are crowded with ripe nematocysts, but the chief region in which young nematoblasts are to be found is the distal region of the body below the level of the tentacles.

As regards the mode of infection it was rather difficult à priori to see how such a small animal as Microstoma could devour a Hydra; but it must be remembered that Hydra is generally observed in an extended condition, whereas Microstoma, except immediately after a meal, is never seen extended to its full size. Even the small Microstoma will readily swallow a fairly large Cyclops or Lycnæid, and these are more awkward animals to digest than a contracted Hydra rubra. If a fasting Microstoma is placed in a watch-glass which contains some small Hydra, it is almost certain in a short time to come into contact with one of them. If the Microstoma comes suddenly against the tentacles of the Hydra it contracts itself immediately, and in this condition it may frequently be killed by the discharged nematocysts. As a rule, however, the Microstoma fixes itself for a short time by its

posterior end in the neighbourhood of the Hydra, and everts its pharynx to its full extent.

The Microstoma then swims over the surface of the Hydra, usually attacking the lower part of its body with its pharynx fully everted (vide fig. 10). The Hydra then usually becomes strongly contracted, and sweeps its tentacles over to the side on which it has been attacked, though under these conditions the tentacles do not grasp the Microstoma, but remain extended almost parallel with its body, and it would appear as though the pharyngeal secretion had a paralysing action on the Hydra. In many cases, after a time, the Microstoma leaves its prey, and in such a case the Hydra does not seem much the worse for the attack, but if the Hydra is of small size, it may be engulfed, and swallowed whole.

To further examine the effect of this pharyngeal secretion I placed some Hydra, which had been kept living in a solution of neutralroth, in a watch-glass with a Microstoma, and it could then be seen that the vacuoles of the ectoderm of the Hydra, which had been stained a pink colour by the neutralroth, took a yellowish-brown colour under the action of the digestive fluid, indicating that the secretion was probably of an alkaline nature, and possibly allied to trypsine. If a Microstoma which contains nematocysts is placed in a watch-glass, and a dilute acid or alkaline added, the nematocysts under the skin are discharged. It would be very interesting to determine how far the Microstoma is capable of using its nematocysts for purposes of attack or defence.

One of the common enemies of Microstoma appears to be Chætogaster, which devours it greedily. One evening I placed six large Microstoma in a watch-glass with two Chætogaster, and by 10 a.m. next morning all had been eaten. In one case which I examined I found a Chætogaster, some time after it had swallowed three Microstoma, had eight large exploded nematocysts in its gut, but the presence of nematocysts in its prey seems to have no deterrent effect upon the Chætogaster.

As regards the mechanism by which this discharge is

effected I think that any muscular or nervous influence in the case of the nematocysts of Microstoma is excluded, and I have never found any trace of a cnidocil-like structure. I am inclined to agree with Grosvenor that the discharge of a ripe nematocyst is only effected when it is brought into a solution of less osmotic pressure. At the time when the nematocysts of the Hydra are ingested by the Microstoma, they lie in a mixture consisting of the pharyngeal secretion of the Microstoma and the partly digested ectoderm cells of the Hydra. When the Microstoma is irritated by acetic acid or an alkaline solution, the ectoderm probably becomes slightly contracted, and the end of the nematocyst is forced between the ectoderm cells, water is taken up, and an explosion follows.

In order to establish a definite proof that the nematocysts of a Microstoma proceed from its prey, there seem to be only three courses open :

(1) To feed the Microstoma upon Hydra in which the nematocysts have been stained intra vitam with some dye, and follow the passage of the coloured nematocysts through the gut under the ectoderm of the Microstoma.

(2) To feed the Microstoma upon some other Cœlenterate which has nematocysts of a different shape to those of Hydra.

(3) To rear Microstoma from the egg without feeding it on Hydra.

In the first case I kept some Hydra in water containing methylene blue, and found that some nematocysts, usually those that were not fully developed, took up the stain with great intensity. I fed some Microstoma on these Hydra, and a Microstoma which was placed with these Hydra at 1 o'clock had a mass of blue in its gut at 5 o'clock. Next day in most cases the colour had faded, and in only one case did I see blue nematocysts actually under the skin. This specimen was mounted, but unfortunately the colour has since, to a large extent, faded.

With regard to the second course, this is the method that has been used with such success by Strethill Wright,

Grenvenor, and Cuénot in their proof that the nematocysts of Æolids were derived from their prey.

This summer I found Microstoma in Hickling Broad in Norfolk. In this Broad, Hydra seems to be fairly rare, but Cordylophora lacustris is very abundant.

Most of the Microstoma I found were absolutely free from nematocysts, but some contained the nematocysts of Cordylophora in the ordinary vacuoles under the skin. Cordylophora contains nematocysts of two kinds.

(1) Oval Barbed Nematocysts. These are of a very characteristic shape. They are more or less egg-shaped structures with one pole smaller than the other, and in profile the one side is slightly convex whilst the other is concave. In the exploded condition the thread passes from the small pole, the swollen base of the thread is rather long and set with numerous barbs which decrease slightly in length in the distal portion.

(2) The Small Oval Nematocysts are rather like the small oval nematocysts of Hydra, and are provided with a short thick thread free from barbs.

I placed a Microstoma, from Hickling, in which I had not been able to find nematocysts, in a watch-glass with some teased-up Cordylophora, and twenty-four hours afterwards I fixed and cut sections of it. The gut still contained portions of disintegrating Cordylophora, but nematocysts were found under the skin, both in the exploded and the unexploded condition.

By feeding a Microstome with Cordylophora which had previously been fed upon Hydra I succeeded in obtaining a mixed infection, the animal having both Cordylophora and Hydra nematocysts under the skin.

Third Course.—This would seem at first sight the most ready method of settling the question, but there are two very great difficulties. During the greater part of the year Microstoma reproduces entirely by budding, and it is only in September in Scotland that I have been able to find sexual individuals.

I have only once found ripe female Microntoma in Loch Lomond towards the middle of September, but the material which I then obtained was unfortunately lost. Even if, however, this difficulty is overcome, I do not believe that trustworthy results can be obtained by this method.

I have found very young Microntoma in which nematocysts were already present, and at first this presented a difficulty to this theory of the derivation of nematocysts, but I do not believe this is insuperable when we consider that nematocyst in the case of an animal which has fed largely on Hydra can be found in almost any tissue of the body. I have found them in the testes (fig. 8), and although I have not yet found them in an ovum, I believe that the yolk-cells might readily carry the nematocysts into the cocoon thus causing the infection of the young forms.

Microntoma giganteum.—This form was described by Hallez, and according to von Graff, the only details given as to the nematocysts are that they are larger and more numerous.

Microntoma rubromaculatum.—This form has only twice been found by von Graff in the Bay of Naples. In his description he says, "Stäbchen fehlen; der Haut dagegen enthält dieselbe Gruppen minutiöser ovaler, starklichtbrechender Körperchen von 0·003 mm. Breite und 0·007 Länge (fig. 16), die ich nach ihrer Ansicht von oben (*a*) und von der Seite (*b*), für Nesselkapseln mit eingeschlossenen kurzen Faden halten muss."

I found this form on one occasion at Naples on some weed from the Mergellina. In the form which I examined there were large cells scattered in the epidermis containing minute rods which stain very readily with eosin. There were also a few small oval nematocysts.

Microntoma papillosum, von Graff.—A single example of this form was found by Claparède at Sarteroe, on the coast of Norway, and figured by him as a Dendrocœl larva. The animal measured ·3 mm. long, and he says nothing of nematocysts, though he figures the long rhabdites. In the

'Zoologischer Anzeiger,' in 1889, Böhmig describes an undoubted Rhabdocœl which he found at Trieste, and which he identifies with Microstoma papillosum. The size of the chains measures 500 μ to 1100 μ. There were no eyes, but adhesive papillæ were present. Nematocysts which he does not figure, were found. From the form of the animal, the appearance of the rhabdites, and the presence of the papillæ, one would feel that it must resemble extremely closely Stenostoma sieboldii.

Stenostoma sieboldii.—Von Graff figures a specimen in which there were present—

(1) Bundles of large rhabdites,

(2) Small oval nematocysts.

It is interesting to observe that the nematocysts lie to the number of 2—5 in common vacuoles under the skin (and apparently in the gut). In the forms which I examined I could not find the small nematocysts, but I was able, by feeding Stenostoma on chopped Eudendrium, to get specimens in which unexploded nematocysts lay in the gut and under the skin.

Kalyptorhynca.

Von Graff, in his Monograph of the Turbellaria, remarks: "In die Kategorie der Nematocysten gehören schliesslich die am Rüssel gewisser Prosbosciden die Stelle der Rhabdites vertretenden Gebilde." He then states that the epithelium of the proboscis in Polycystis mamertinus and nægellei contains almost identical "eiformige gebilde," which he considers to be nematocysts, and which he believes show their homology with true rhabdites through the presence of intermediate forms.

Von Graff was unable to detect the expulsion of a thread in these structures, and I believe that they can only be regarded as rhabdites resembling those on the rest of the body. In sections stained with methyl-blue-eosin these structures on the proboscis and the rhabdites of the skin take up a similar bright red colour (figs. 13 and 14).

As regards the function of the proboscis in these forms there seem to be two divergent views: (1) Hallez regards the proboscis mainly as an organ of prehension for seizing the animal's prey, and in his account of Gyrator notops the following passage occurs: "Si l'on place sur le porte-objet d'un microscope quelques Gyrator, avec des Cyclops, on ne tarde pas à voir, a la première rencontre, le Gyrator devaginer sa trompe et fixer lentement sur sa victime l'epithelium adhésif de son organe de préhension." Hallez describes rhabdite-like structures in the proboscis, to which he is inclined to attribute an adhesive function. (2) This view is strenuously denied by von Graff in his monograph. He asserts that the proboscis functions only as a tactile organ. If we regard the proboscis as a purely tactile organ it is very hard to explain its enormous muscular development in such forms as Polycystis goettii, nægelii, Gyrator, etc. The base of the proboscis consists of an enormous mass of muscle to which are attached long powerful retractor muscles. I have myself seen both Polycystis goettii and Polycystis nægelii capture Copepods with their proboscis. In one case in which a Polycystis nægelii had captured a large Copepod in this manner, it bent round and started swallowing the posterior thoracic feet, which seemed paralysed, although the violent movements of the gut and the anterior appendages showed that the animal was still alive.

Alloiocœla.

Allostoma monotrochum.—As far as I know only three specimens of this form have been found by von Graff, in 1879, in Trieste. He describes it as possessing oval bodies in the skin from 0·003—0·004 mm. long, with the appearance of a coiled spiral thread which, however, he never saw ejected.

Polyclads.

I have not been able to examine any of the Polyclads which have been described as possessing nematocysts, as they are unfortunately extremely rare.

Anonymus virilis has only twice been found by Lang in the Bay of Naples. The nematocysts are described as oval structures. They are formed in the parenchyme and pass along specialised tracts to the surface. They have never been seen discharged.

Stylochoplana tarda.—This form, as far as I know, has only once been found by von Graff at Trieste. It is interesting to observe that it only differs from Stylochoplana fusca by its slightly smaller size, sluggish habits, and the possession of nematocysts. The nematocysts have not been figured, but they are said to be over the structures ·01 mm. long with a thread ·015 mm. long, and a base covered with spiral rows of short spines ·009 mm. long. In this case it is very tempting to suppose that the nematocysts, which are of a very common Cœlenterate type, were ingested with the food, and that this species is really identical with Stylochoplana fusca.

Conclusions.

There are only three possible views as regards the presence of nematocyts in Turbellaria :

(1) It might be said that they are absolutely homologous structures with the nematocysts of the Cœlenterates, and to be an indication of the very close relationship between these groups (a theory that is put forward in a recent text-book). There are, however, several difficulties to the acceptance of this view. In the first place no one has yet been able to trace the development of a nematocyst in a Turbellarian nematoblast. And secondly, it would be very difficult to show Cordylophoran affinities in the Microstoma from Hickling as compared with the Hydroid affinities of those of the neighbouring Sutton Broad.

(2) The presence of nematocysts might be regarded as a case of convergence, and if this theory were accepted it must be generally admitted that we have here the most remarkable and repeated examples of convergence in the animal kingdom. In this case again the experimental evidence, in which it is shown that Microstoma changes its type of nematocyst with its change of food is fatal.

(3) The one remaining possibility is the transference of the nematocysts of a Cœlenterate to the animal which preys upon it. The theory which was first established by Strethill Wright for Æolids. It is clear from what has been written above that this last view must be accepted. Since it has been shown that—

(*a*) The nematocysts of Microstoma lineare are normally derived from the Hydra upon which it feeds.

(*b*) If the Microstoma is fed upon Cordylophora, Cordylophora nematocysts are found under the skin.

(*c*) The nematocysts of Stenostoma sieboldii are derived from the Cœlenterata on which it feeds.

The same process probably occurs in the other Turbellaria with the possible exception of Anonymus virilis, and therefore the presence of three nematocysts in a Turbellarian offers no ground for the generally accepted homology between Nematocysts and Rhabdites.

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EXPLANATION OF PLATE 14,

Illustrating Mr. C. H. Martin's paper on "The Nematocysts of Turbellaria."

Fig. 1.—An oval barbed nematocyst with two small oval nematocysts lying in a common vacuole under the skin of a living Microstoma lineare. Zeiss apoc. 2 mm. + 6 comp. oc.

Fig. 2.—Small oval nematocyst from a living Microstome. Outline same magnification as Fig. 1.

Fig. 3.—Cylindrical nematocyst in vacuole in skin of Microstoma lineare.

Fig. 4.—Part of a transverse section through Microstoma lineare fed on Hydra rubra.

a. Oval barbed nematocyst in gut cell.
b. Under the skin.
c. A cell from the wall of the vacuole lying above the nematocyst.

FIG. 5.—Part of a transverse section through Microstoma lineare, showing nematocysts, some of which lie in vacuoles in the cells of the gut, while others have been carried through into the body cavity. *Ep.* Epidermis. *B.C.* Body cavity.

FIG. 6.—Nematocyst surrounded by Phagocytes lying in the body cavity. Transverse section of Microstoma.

FIG. 7,—Nematocyst lying under the skin. The wall of the vacuole has not yet been passed. Transverse section of Microstoma.

FIG. 8.—Nematocyst lying in the testis of Microstoma lineare. *Sperm.* Spermatocyte. *Te.* Testis.

FIG. 9.—Exploded nematocyst lying in the vacuole under the skin. Transverse section of Microstoma.

FIG. 10.—Outline of Microstoma lineare, showing the fully expanded pharynx when collecting Hydra. *Ph.* Pharynx.

FIG. 11.—Exploded Cordylophora nematocyst from the skin of Microstoma lineare. Only part of the thread is drawn.

FIG. 12.—Exploded Cordylophora nematocyst lying in a vacuole under the skin. From a transverse section of Microstoma lineare.

FIG. 13.—Longitudinal section through the proboscis of Polycystis nægelii. 4 mm. + 4 comp. oc.

circ.musc. Circular muscle. *Prob.* Proboscis. *ret.musc.* Retractor muscles. *Sph.* Sphincter muscles.

FIG. 14.—Part of epidermis of the proboscis of Polycystis nægelii from a transverse section. 2 mm. + 6 comp. oc.

a. Rhabdites in epidermis of proboscis.

b. Rhabdites in epidermis over the body.

FIG. 15.—Part of a crushed Stenostoma sieboldii, showing Tubularia nematocyst in vacuole.

Ne. Nematocyst. *Rh.* Rhabdite.

Doridoeides gardineri: a Doridiform Cladohepatic Nudibranch.

By

Sir Charles Eliot,
Vice-Chancellor of the University of Sheffield;

and

T. J. Evans,
Lecturer in Zoology in the same University.

With Plates 15 and 16, and one Text-figure.

The nudibranchiate mollusc which forms the subject of this paper presents an unusual combination of characters. It has the external appearance of a Dorid, except that it has no gills, but its digestive organs are arranged on the cladohepatic plan. It was originally described by Sir Charles Eliot as Doridomorpha gardineri in Mr. J. Stanley Gardiner's 'Fauna and Geography of the Maldives and Laccadives, Nudibranchiata,' pp. 544-5. Professor Bergh, however, has pointed out ("Ueber clado- und holohepatische nudibranchiate Gastropoden," 'Zool. Jahrb.' Bd. xxiii, Heft 6, p. 740) that Doridomorpha was used in 1832 by Audouin and Milne Edwards. The animal to which they gave the name cannot now be identified, but it is unfortunately incorrect according to the laws of nomenclature to apply it to a new form and Doridoeides is now proposed as a substitute of equivalent meaning.

The original description was made from a single specimen obtained by Mr. Stanley Gardiner in the Fiji Islands

(Rotuma). It was small (6·5 mm. by 5·5) and much hardened so that a satisfactory anatomical investigation was impossible. Mr. Crossland and Sir C. Eliot both examined it and could find no trace of a blood gland or of a second spermatotheca but, as those organs are characteristic of the Doridida, and as Professor Bergh had described them as present in Doridoxa, an externally similar gill-less doridiform animal. they did not venture to regard their absence as certain, and tentatively referred Doridomorpha to the Doridoxidæ, adding that it might possibly prove to be the type of a new family. An examination of more numerous and better preserved specimens obtained by Mr. Stanley Gardiner on his last journey to the Seychelles shows that this is the case. The second spermatotheca and blood gland are really absent and the animal has a ramified liver. It is, therefore, despite its general resemblance to Doridoxa, not very nearly allied to it and cannot be placed in the same family.

Doridoeides gardineri, sp. nov.

= Doridomorpha gardineri Eliot (in Nudibranchiata of J. Stanley Gardiner's 'Fauna and Geography of the Maldives and Laccadives,' vol. ii., part i, 1904).

Eleven specimens labelled Coetivy and preserved in formol. Coetivy is the southernmost island of the Seychelles group, and Mr. Gardiner's notes state that the nudibranchs obtained there were "all from the reefs, which differ from those of the Chagos archipelago in being almost completely covered with Zostera." It is probable that the animal adheres to the leaves of the Zostera and harmonizes with them in colour.

The natural shape seems to be flat and nearly circular, but the preserved specimens are bent in various ways and have the edges turned inwards. One which seems to have kept its form fairly well is 10 mm. long, 9 broad and 2 high. These are the average dimensions of the consignment. In the individual mentioned the foot is 6 mm. long and 3 broad, but

was evidently much wider in life as the margins are bent and rolled up. The free part of the mantle starting from its junction with the foot is 3·5 wide. It can be seen from the outside that about 2·5 mm. of this space are filled with dark internal organs and that only about 1 mm. of it corresponds to the mantle margin generally found in Dorids. The genital orifice is about 4 mm. from the anterior end, and the anus is about 2 mm. behind it.

The animals are of a greyish-green colour, a little darker in the centre where the internal organs show through, and lighter at the edges where there are none. Practically the coloration is uniform, though some specimens are lighter than others and the under side is usually rather lighter than the upper.

To the naked eye the dorsal surface appears to be smooth, but under a low power can be seen to be covered with small warts of various sizes, sometimes connected by an irregular reticulation and with minute pits between them. There is no median ridge and no trace of a branchial opening. The integuments are devoid of spines. The dorsal epidermis is thick and consists of several layers of cells: it is profusely pitted with mucus glands (fig. 2, *b*) and in many places rises into folds (fig. 2, *a*). On the under surface of the mantle and on the foot the epidermis is thin, but the foot is highly glandular. It contains both epidermal glands and subepidermal glands with granular contents and communicating with the exterior by long necks.

The rhinophores are completely retractile. Their pockets are simple holes without sheaths, visible to the naked eye; but in the sections it can be seen that the margins are slightly raised. As preserved, the pockets are often closed. When retracted the rhinophores often exhibit a few (6—7) strong transverse wrinkles or furrows, and these were also present in the specimen obtained at Rotuma. But they are probably not real perfoliations since they are absent when the rhinophores are completely exserted. In such cases the outline is even and cylindrical. There are no oral tentacles and nothing

which can be called a head. In some specimens the mouth is a simple orifice above the foot, but in others there is a sort of snout. It would seem, however, that this protrusion is due to artificial causes and is not a natural and permanent structure. There are no lamellæ on the under side of the mantle as in Phyllidia, etc., but it is uneven and in some cases presents ridges and bulges probably caused by the hepatic follicles. The foot is large, with ample expanded margins. The anterior margin is straight and not grooved.

When the internal cavity is opened, the central nervous system (fig. 3) is seen lying on the top of the œsophagus and surrounding it. There is no trace of a blood-gland. The cerebro-pleural ganglia (fig. 3, *a*) are rather large and elliptical. Externally they show no division but a section indicates that the ganglionic mass is of dual origin and divided internally by a constriction into two nearly equal ellipsoids. The pedal ganglia (fig. 3, *b*) are also elliptical and lie below and rather behind the cerebro-pleural. The buccal ganglia (fig. 3, *d*) are rather large, close to the pedal ganglia and also close to one another. No gastro-œsophageal ganglia could be found and no otocysts. The eyes are black and lie near the base of the rhinophores on the olfactory ganglia (fig. 3, *c*), the optic and olfactory nerves being apparently fused. This arrangement is unusual, but something similar may be seen in Bergh's figure (Malac. Unters. in Semper's 'Reisen,' Heft. xv, pl. lxxi, fig. 17) of the nervous system of Tritonia (Candiella) plebeia where the optic and olfactory nerves are joined for a considerable distance and separate only in their upper portion. The pigment layer of the eye lines a cup formed of a few large retinal cells, from which fibres run into the olfactory nerve at the base of its ganglion.

The jaws are yellowish but not deeply pigmented in any part, moderately convex, not very broad, united at the top by a hinge, and provided with short processes. The edge (fig. 4) is armed with a row[1] of very distinct projections with spatu-

[1] In the specimen described by Sir C. Eliot and Mr. Crossland two rows of broad denticles were found on the jaws, which were relatively wider, and the

late tips. Near the end of the row they appear thin and filamentous, possibly because they have become worn or folded on themselves. The radula (fig. 5, *a*) consists of about twenty-six rows, one or two of which are imperfectly developed and shadowy, with a constant formula of 4.1.4.[1] The teeth are neatly arranged in a close-fitting mosaic. The base of the large median tooth, which is arched and hollowed out behind, is nearly twice as broad as all the four laterals together. It bears a single cusp, large and only slightly bent downwards (fig. 5, *b*). The first lateral (fig. 5, *c*) is about three fifths the length of the median tooth but only a quarter of its breadth, with a single hamate cusp. The second and third laterals are similar but slightly smaller and more bent. The outermost tooth (fig. 5, *d*) is considerably smaller but more erect and stands up conspicuously at either end of the row. There are two salivary glands; their distal portions are expanded and spread over the genitalia and stomach. The left is much larger than the right. The remaining portion of each gland is band-like and terminates in a long thin duct which passes through the nerve collar and enters the posterior part of the buccal mass. Several glands, probably ptyaline, open into the buccal cavity, but they are embedded in the wall of the cavity and are not visible on its outer surface.

The œsophagus (fig. 6, *a*) is not long, and leads straight into the stomach, which is divided into two parts (fig. 6, *b* and *c*) by a constriction more marked on the right than on the left side. There is no structural difference in the walls of these two divisions, and neither contains any spines or plates, but as the hepatic ducts all open into the second division, the first should perhaps be regarded as a dilatation

radula, which was disarranged and in confusion, was estimated to contain 13 teeth in each row (i.e. 6 + 1 + 6). But these differences cannot be regarded as specific unless shown to be certain and constant. In Tritonia the number of rows of denticles in the jaw varies in several species, and the formula of the radula in the original specimen was doubtful. The shape of the teeth is the same.

[1] See footnote above.

of the œsophagus. Neither contained any solid food in any specimen. The intestine (fig. 6, *d*) issues from the mid-dorsal surface of the second division and, after describing a broad loop backwards and downwards, runs to the anal opening, which is an inconspicuous papilla on the right side, lying below the mantle edge and just at the point where it joins the body. The loop of the intestine bears a single longitudinal ridge resembling the typhlosite found in Lumbricus.

The stomach receives three hepatic ducts: one on the right (fig. 6, *e*), close to the exit of the intestine; one on the left (fig. 6, *g*), not quite opposite to it, but a little posterior; and one behind (fig. 6, *f*). The posterior and left ducts bifurcate close to the stomach and then ramify into branches composed of follicles which are not only found in the body cavity but enter the body wall and dorsal integuments, extending to within a short distance of the mantle brim. The arrangement and extent of the right duct is essentially the same, but the follicles are developed more luxuriously on this side than on the left, and the bifurcation is less clear, although the duct runs in two directions, backwards and forwards. The right and posterior branches anastomose, but though the right and left branches almost meet anteriorly they seem not to communicate, nor do the posterior and left branches. All three branches consist of variously shaped follicles communicating with one another, so as to offer a continuous passage but not forming a cylindrical tube except in the main ducts. For some distance from the point of entry into the stomach the walls of the main duct bear folds which dovetail into one another in the middle of the lumen and form a valve or strainer.

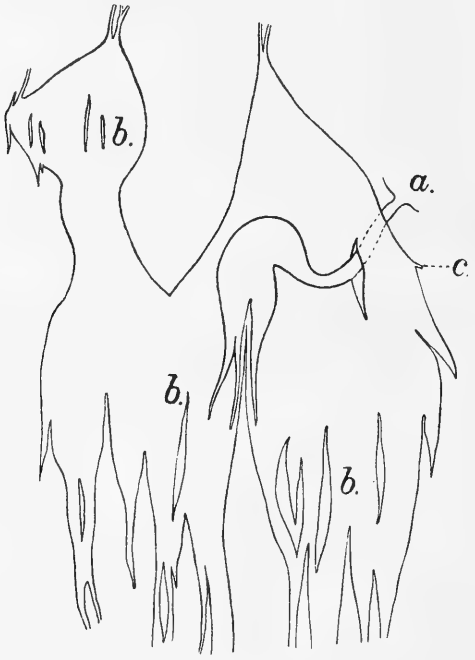
The cells which line the hepatic lobules are columnar or cubical and highly granular. Some are in a distended condition, others are attached to the wall of the lobule only by a strand or are free in its cavity. It would seem, therefore, that some of the liver cells are excretory in function, and are dropped into the follicle as they become extended with excreted material.

The heart (fig. 7) lies somewhat to the right of the median line. The walls are thin, and not strong. From the ventricle issue the anterior and posterior aortas, but the aortic system is not much developed, as is perhaps natural in an animal that has no gills. The arteries are thin, and do not extend beyond the level of the genital orifice in front and of the anus behind. The musculature of the ventricle also is feebly developed. The venous system is extensive and the veins are provided with valves (fig. 8) not only near the heart but in parts far from it, e. g. throughout the length of the lateral veins and venules and between the blood lacunæ of the foot. The auricle (fig. 7, *c*) has a large extension (fig. 7, *d*) on the left, enclosed by a corresponding extension of the pericardium; it adheres to the anterior part of the ventricle, the line of adhesion being zigzagged, and on the right it is attached to the wall of the pericardium.

The kidney (see text-figure) is a shallow chamber lying on the surface of the hermaphrodite gland, and sending downward prolongations between its follicles. In front it bifurcates like the hermaphrodite gland and is divided behind into a number of irregular tubes by the dorso-ventral muscles and the projecting genital lobes. The renal opening (*c*) is minute and near the anus. From the reno-pericardial opening (*a*) a tube passes dorsally through the substance of the kidney and dilates in the median line into a flattened vesicle. Posteriorly this sub-divides into three fine ducts, each opening into the kidney by a minute aperture. The wall of the kidney is formed by a layer of large, clear, cubical cells, the nucleated protoplasmic portion of which is limited to a small quantity at the base of the cell.

The genitalia (fig. 9) are large, and occupy most of the right-hand side. The hermaphrodite gland (fig. 9, *a*, and fig. 10) consists of a single undivided mass of roughly triangular shape, bifurcating in front so as to enclose the ampulla and the spermatotheca in the fork. It is traversed by branches of the liver, which enter it from below, by the dorso-ventral muscle bands (fig. 10, *d*) and by various pro-

longations of the blood lacunæ of the foot. The kidney (fig. 10, *e*) also sends prolongations into its upper surface. It presents a series of lobes the outer layers of which are formed by masses of ova (fig. 10, *b* and *c*). Within each lobe is an ample loculus (fig. 10 *a*), larger than the whole mass of ova, containing spermatozoa in all stages of develop-



Kidney of Doridoeides, seen from the dorsal side (× 22). *a*. Reno-pericardial opening. *b*. Gaps for passage of dorso-ventral muscles and protruding lobes of hermaphrodite gland. *c*. Renal opening.

ment. But there is no symmetrical arrangement of ovarian follicles round a central male follicle.

The hermaphrodite duct (fig. 9, *b*) is thin and short, but swells out into an unusually large pear-shaped ampulla (fig. 9, *c*), which again contracts into a thin tube. After a short straight course this tube (fig. 9, *f*) bifurcates. The male portion (fig. 9, *d*) runs forward and describes a com-

plete loop, after which it first dilates into a prostatic portion (but without any trace of a separate prostate gland) and then contracts into a muscular portion, terminating in a thin conical glans penis (fig. 9, *e*). No trace of spicules or other armature was found in this or any part of the genitalia. After the main bifurcation dividing the male and female branches, the female branch runs backwards for a little distance as a short tube and then itself bifurcates. A short duct leads to the spermatotheca (fig. 9, *g*), which is large, globular, and single, no trace of a second receptaculum seminis being found. The walls of the spermatotheca are thick, and produce a secretion. In some specimens small clumps of spermatozoa are embedded in this secretion. In others all the spermatozoa form a central mass in the main cavity of the spermatotheca. It is possible that the secretion serves to form small packets of spermatozoa or spermatophores. The spermatotheca communicates by a long thin duct with the vaginal opening (fig. 9, *h*) which lies at the base of the penis. The other division of the female branch enters the mucus [1] gland (fig. 9, *m*), enclosing the albumen [1] gland (fig. 9, *l*), which is smaller. The mucus gland communicates with the exterior directly by a slit-like irregular aperture (fig. 9, *i*) which lies a little behind the other orifices and is much larger than they are. Only spermatozoa are to be found in the ducts and in the spermatotheca. There are no ova except in the hermaphrodite gland, where they are in process of ripening or nearly ripe.

In all the specimens examined microscopically were found scattered cells which do not seem to form part of the essential bodily structure. They are large and rounded in outline, with vacuolated contents and a large round nucleus. They occur chiefly in the connective tissue spaces, in spaces hollowed out in the dermal muscle layers and among the epidermal cells. The fact that they are absent from the cavities of all the internal organs and from the lacunar blood spaces, and

[1] The functions of these glands are presumably as indicated by their names, but it is not easy to say which is which.

that they are limited to the dorsal regions of the body which are of a deeper green than the ventral surface, suggests that they are of the nature of Zoochlorellæ or symbiotic algæ.

We see no reason to doubt that this animal is specifically the same as that previously described under the name Doridomorpha gardineri. As noted above there are some discrepancies (which are, however, explicable) in the descriptions of the buccal parts, and the drawings of the teeth now published do not give quite the same impression as the simpler diagrams made by Mr. Crossland. But on comparing these teeth with those of the original specimen, which have been preserved, we can find no essential difference in shape.

The generic characters may be extracted from the above description and formulated as follows:

Doridoeides, gen. nov.

Form flat, doridiform. Dorsal surface smooth: no appendages of any kind except two rhinophores retractile into pits. No oral tentacles. Foot and mantle margin wide: anus lying between them on the right hand side. No blood gland. Heart somewhat to the right of the median line. Jaws distinct and denticulate. Radula narrow, consisting of a large strongly cusped central tooth and a few (4) laterals. Stomach without plates or spines. Liver system cladohepatic, entering the stomach by three ducts and extensively ramified in the mantle margin. No cnidocysts. Kidney not much branched. Hermaphrodite gland a single undivided mass: one spermatotheca: three genital orifices: no armature in the genitalia.

These characters do not agree with those given for any recognized family of nudibranchs. Superficially Doridoeides resembles Doridoxa (Bergh, 'Ingolf Expedition,' vol. ii, 1900, 'Gastropoda nudibranchiata,' pp. 15-19), but Bergh states that this latter has (1) a large blood gland, (2) a holohepatic liver opening into the stomach by a single opening, (3) a spermatocyst, although "its relation to the sperma-

totheca could not be determined." The affinity between the two genera is therefore not close. In structure Doridoeides is more nearly allied to Pleuroleura (= Dermatobranchus) but the general habitus and the configuration of the anterior parts and rhinophores is different, and unless intermediate links are discovered it is not clear that Doridoeides is either the ancestor or descendant of Pleuroleura. It must therefore be made the type of a new family Doridoeididæ, the characters of which are at present the same as those of the genus, but the absence of oral tentacles, the narrowness of the radula and the denticulation of the jaws are probably not of more than generic importance. The family belongs to the Cladohepatica. Its only anomalous characters are the doridiform shape (which is really not very different from that of the Pleurophyllidiidæ) and the presence of three genital openings. But, as Brüel observes ('Geschechts- und Verdauungs-organe von Caliphylla mediterranea,' Halle, 1904), although it is commonly stated that all the Æolididæ are simply diaulic, the data available do not justify so comprehensive a statement. It is nevertheless true that Doridoeides presents the arrangement of the genitalia which is typical of the Doridida, except that the second spermatotheca cannot be found. Although in that group the hermaphrodite gland usually takes the form of a layer spread over the liver, yet it is a separate mass (or masses) in Bathydoris, Alloiodoris, Doridoxa and Trevelyana.

Doridoeides is thus an annectant form connecting the Holohepatica and Cladohepatica, but having the essential characters of the latter, and its systematic position can hardly be fixed without reference to our general ideas respecting the phylogeny of the Nudibranchiata. The most definite view respecting this phylogeny is that put forward by Pelseneer ('Recherches sur divers Opisthobranches,' 1894). According to it the Tritoniidæ are allied to the Pleurobranchidæ, especially Pleurobranchæa. The Tritoniidæ are the common source of the Doridioidea and Æolidioidea, which

represent two lines of development in two different directions. The Elysioidea are derived from the Æolidioidea. Bergh has frequently expressed the opinion that our knowledge of the Opisthobranchiata is not sufficient for the formulation of any phylogeny, and his own views are so tentatively and undogmatically expressed that it is difficult to summarise them. In his 'System der Nudibranchiaten Gasteropoden' (1892) he appears to regard the Nudibranchiata as diphyletic, p. 996 ("Durch die Ascoglossen knüpft die eine Gruppe der Nudibranchien an die Aphysiaden und die Bulliden an, die andere durch die Pleurobranchiden wieder an diese letzteren"), the Æolids being nearest to the Ascoglossans, and the Tritoniidæ being derived from the Æolids by gradual reduction of the hepatic ramifications, as seen in Bornella, Dendronotus, and Scyllæa. In his article "Ueber clado- und holohepatische nudibranchiate Gastropoden" ('Zool. Jahrb. System.,' 1906, pp. 739—742), while still maintaining this view of the Tritoniidæ, he regards Tritonidoxa, Doridoxa, and Bathydoris as bridging over the interval between the Tritoniidæ and the Dorids or Holohepatica. It is not clear what is the relationship of the Holohepatica to the Pleurobranchidæ on this hypothesis, and it seems to be implied that the Æolids, which are a highly specialised type, lose their peculiarities and pass into the Tritonids, which are a comparatively generalised type, and that the Tritonids then develop a new highly specialised type, the Dorids. We find it hard to accept this view without stronger evidence than is forthcoming. The branching of the liver may disappear in some cases,[1] and the animal which forms the subject of this memoir might be regarded as a derivative of the Æolidiidæ which has retained its cladohepatic system and adopted a doridiform shape. But a consideration of the whole series of forms now known (many of which have been described only in the last ten years) inclines

[1] E. g. in Pseudovermis, and Trinchese states that in the larva of Lomanotus eisigi the æolidiform and cladohepatic characters are much more marked than in the adult.

us to believe that the holohepatic condition is the more primitive, and the cladohepatic condition derived from it, though it may make its appearance very early in the pedigree of the nudibranchiata. Further, if it is admitted that a comparatively unspecialised group (such as the Tritoniidæ) connects two highly specialised groups (such as the Æolidiidæ and the Doridídæ), the origin of the whole series is probably to be sought in or near the unspecialised group, and we therefore think with Pelseneer that Tritonia,[1] or rather some extinct allied form must be the ancestor of both the Holohepatica and Cladohepatica, and likewise nearly allied to the Tectibranchs.[2] Pleurobranchæa is certainly the Tectibranch which most nearly resembles the Holohepatica, but it does not follow that it is necessarily their direct ancestor, for the characters of the Pleurobranchidæ appear to be due to the shell being enclosed by the integuments, the asymmetrical ctenidium remaining; whereas in many nudibranchs, at any rate, the shell is rejected in the larval stage, not enclosed, no ctenidium is formed, but symmetrical respiratory organs of various types are developed instead. Nothing except the fact that the more primitive nudibranchs, as far as they are known, seem nearly allied to one another, renders it improbable that more than one type of larva may have adopted this method of development by rejection of the shell and symmetrical growth.

Are the more primitive nudibranchs those with or those without special gills? It will be well to review the principal gill-less forms more or less allied to Tritonia. It may be premised that all the Doridídæ appear to have pallial respiration (as also the Pleurobranchidæ) which is merely supple-

[1] Tritonia itself may have developed special features, such as its oral veil, tentacles, peculiar rhinophores, and branchiæ.

[2] This is without prejudice to the question of the derivation of the Elysioidea from the Ascoglossa. It seems to us possible that the Nudibranchiata (like the shell-less Pulmonata) may be polyphyletic, but that, if so, it is more likely that the Elysioidea have a different origin from the Æolids than that the Æolids have a different origin from the Dorids. But Myrrhine to some extent connects the Æolids and Elysioidea.

mented not replaced by the action of accessory gills. It may even be said that the Æolids and many other cerata-bearing Cladohepatica have nothing but pallial respiration, for the cerata and the hepatic ramifications which they contain are not so much special respiratory organs as a special disposition of other organs arranged so as to facilitate pallial respiration. But the following genera have neither this arrangement nor accessory gills.[1]

Tritonidoxa, Bergh.[2] Similar to Tritonia in all respects except that it has a broad, undulating dorsal margin without any trace of branchial tufts. Dorsal surface smooth. Size (32 mm.) moderate.

Tritoniella, Eliot.[3] Similar to the last genus, and like it resembling Tritonia in most peculiarities, but the dorsal margin, which is wide, bears simple unbranched prominences and not foliaceous tufts. The back bears ridges. Rather large (63 mm.).

Doridoxa, Bergh.[4] Doridiform in appearance and holohepatic. Blood gland and two spermatothecas. But there are no branchiæ, the anus is lateral, jaws are present, the

[1] We have not taken into consideration Pseudovermis or the Hedylidæ. The former appears to be a retrograde Æolid. The systematic position and relationship of the Hedylidæ are not clear. The same may be said of Timorella (Bergh, 'Siboga Exp., Opisth.,' p. 241, 1905).

[2] "Ueber clado- und holohepatische nudibranchiate Gastropoden," 'Zool. Jahrb. Syst.,' 23 Band, 6 Heft, 1906; and "Marine Investigations in S. Africa," vol. v, part i—Opisthobranchiata, pp. 86—88, in 'Trans. S. Afr. Phil. Soc.,' vol. xvii, 1907.

[3] Eliot, 'National Antartic Expedition (Discovery) Nudibranchiata,' 1907, pp. 5—11. Tritoniella seems generically distinct from Tritonidoxa since it has dorsal ridges and prominences on the dorsal margin. If the two genera are regarded as synonymous the name Tritoniella has priority. The description was published on March 5th, 1907, and the chief characters had already been named June 9th, 1905 (Eliot, 'Trans. R. Soc. Edinburgh,' vol. xli, part iii, No. 22, p. 525). The description of Tritonidoxa appeared on March 14th, 1907, the chief characters having already been named by Bergh in 'Zool. Jahrb. Syst.,' 23 Band, 6 Heft, 1906.

[4] In 'Danish Ingolf Expedition,' vols. 2, 3, 1900, "Nudibranchiate Gastropoda."

radula is about 36+1+36 and the hermaphrodite gland is separate from the liver mass. Small (12 mm.).

Heterodoris, Verrill.[1] Imperfectly described. Form like Triopa; no gills; back bearing papillæ and a longitudinal crest; rhinophores retractile; anus lateral (?). Radula 168. 0. 168. Jaws? Genitalia? Moderate size (28 mm.). This little-known form is possibly related to the Polyceradæ.

Charcotia, Vayssière.[2] Resembles the Tritoniidæ in form. Back tuberculate. Rhinophores not perfoliate and not retractile. A membranous expansion round the mouth. Jaws present. Radula 1+1+1. Liver divided into three glandular masses, but not passing (it would seem) outside the main body-cavity. Hermaphrodite gland mingling with the posterior liver mass. Small (14 mm.).

Phyllirhoidæ. A family of pelagic nudibranchs with no appendages except rhinophores. Jaws present. Radula varies from 1+1+1 to 11+1+11. Three or four hepatic coeca, not ramified. Hermaphrodite gland in several separate lobes.

Dirona,[3] MacFarland. Æolidiform in appearance, but the papillæ do not contain hepatic diverticula, and there are no branchial tufts. Liver trilobed but solid, wholly contained in body-cavity and not ramified. Hermaphrodite gland consisting of several separate lobes. Anus far back on right side. Jaws: radula 2+1+2. Small (19 mm.).

Pleuroleura, Bergh[4] (=Dermatobranchus, v. Hasselt). Allied to Pleurophyllidia, from which it differs only in having no branchial lamellæ beneath the mantle. Shape quasi-doridiform, but rhinophores and head parts modified, perhaps in connection with burrowing habits. Jaws: radula varying from 4+1+4 to 41+1+41. Liver ramified, and

[1] 'Trans. of the Connecticut Acad.,' vol. 2, 1882, pp. 548-9.

[2] In 'Expédition antarctique française du Dr. J. Charcot,' "Mollusques nudibranches et Marséniadés," 1906, pp. 27—31.

[3] See Cockerell and Eliot, "Notes on a Collection of Californian Nudibranches," 'Journal of Malacology,' 1905, pp. 45—48.

[4] See especially Bergh, "Die Pleuroleuriden," in 'Zool. Jahrb. System,' 3 Band, 3 Heft, 1888, p. 348 ff.

penetrating to mantle margin; cnidocysts present. One spermatotheca: hermaphrodite gland a single mass, separate from the liver. Most of the species are small. Some attain a length of 35 mm.[1]

Gill-less molluscs are on the whole smaller than the corresponding forms with gills, and the genera less numerous in species and probably in individuals;[2] but except for this the presence or absence of gills seems to make no difference, similar forms being found with and without them. A remarkable instance of this is seen in the Docoglossa. Acmæa has a ctenidium only; Scurria a ctenidium and a circle of accessory pallial branchiæ; Patellidæ the circle of pallial branchiæ only; Lepetidæ and Bathysciadium no branchiæ whatever. Yet all these forms seem to lead the same kind of existence, and to thrive equally well. Similarly in the Pteropoda we find Dexiobranchæa with only a lateral gill, corresponding to a ctenidium; Pneumoderma with this lateral gill and secondary terminal gills as well; Notobranchæa with these secondary branchiæ only; and Clione with no branchiæ at all.

In the other Opisthobranchiata the combination of a ctenidium with accessory branchiæ has not been recorded, but we find, though not within the limits of one family, Pleurobranchæa with a fully developed ctenidium, Tritonia with no ctenidium but with secondary pallial branchiæ, and Tritonidoxa with no branchiæ at all. The ctenidium in the Pleurobranchidæ does not show any sign of becoming vestigial[3] preliminary to its disappearance, and the suddenness of the change from the Tectibranchs to the

[1] On the hypothesis that the Elysioidea are derived from the Lophocercidæ, it is noticeable that gill-less forms make their appearance near what may be supposed to be the point of origin. In the Limapontiidæ the liver is not much ramified, and there is no trace of gills. In Elysia a number of ridges radiate from the pericardium, but hardly form branchial lamellæ; the liver is ramified in the wide lateral wings, much as in Doridoeides.

[2] But the Pteropod Clione is found in large shoals.

[3] In the Peltidæ (which appear not to be derived from the Pleurobranchidæ) the gill is small and simple.

Nudibranchs is perhaps explicable by the fact that the larvæ of the latter reject the shell before the gill is formed.

Now the absence of gills is certainly not a primitive condition in mollusca, and in many groups the gill-less forms are obviously specialized or degenerate. But the Nudibranchiata are admittedly derived from the Tectibranchiata by suppression of the ctenidium and, as parallel forms are found with and without secondary gills among the less specialized nudibranchs (e.g., Tritonia and Tritonidoxa), it may be that the gill-less forms remain as a record of the first weak effort to develop a new type which greatly increased in strength and variety by the acquisition of secondary branchiæ. Tritonia and Doris are clearly much more successful types than Tritonidoxa and Doridoxa and their superior respiratory apparatus may be the cause. On the other hand if the forms with gills are supposed to be the earlier, it is not obvious why so many families have lost their gills. The special conditions of pelagic and fossorial life might explain their disappearance in Phyllirrhoe and Pleuroleura (though Pleurophyllidia which has branchial lamellæ and is much richer in species than Pleuroleura, is also fossorial), but there is no obvious specialization about the other forms. Tethys (with pallial branchiæ) and Melibe (without them) are very similar forms, and in some respects Melibe seems the more archaic of the pair, since it possesses jaws which Tethys has lost. The question can be settled definitely only by the discovery of forms more primitive than those now known (that is to say, clearly intermediate between the Tritoniidæ and the Tectibranchs), and we merely wish to indicate the shape it assumes in the light of the interesting new genera recently discovered. Tritonidoxa and Tritoniella are little more than Tritonias without branchiæ: Doridoxa is a real connecting link, a Tritonid with many of the special characters of the Doridididæ. Dirona is a Tritonid with papillæ on the back, but the liver though lobed is not ramified: Charcotia also seems to

connect the Tritonidæ with some of the Æolidioidea but its affinities are less certain.

Doridoeides should probably not be regarded as a very primitive form. Its genital ducts are triaulic and the liver is elaborately ramified. Still, it obviously marks a stage when the characters of the Æolidiidæ, Tritonidæ and Doridæ could be combined in one form. The doridiform shape is probably not important morphologically: it occurs in very diverse families of Gastropods (besides nudibranchs, it is found in the Pleurobranchidæ, Oncidiidæ, Lamellariidæ and many Chitons) where the shell is absent or small, and it is probably largely due to mechanical reasons. The structure of the rhinophores is more significant. The jaws and radula are interesting for they show that these organs are practically the same in Bathydoris, Doridoxa, Tritonids, Doridoeides and Pleuroleura, the chief difference consisting in the width of the radula. Narrow radulas are characteristic of the more specialized Cladohepatica, except Antiopella (Janus), but also occur in some species of Tritonia (Candiella) and Pleuroleura. As Doridoeides is small, and no other form of the same structure is known, it may be presumed that Nature's experiment in making this combination of characters has not proved a success. The large number of æolidiform nudibranchs seems to show that the cladohepatic arrangement without accessory branchiæ answers better in active animals with dorsal appendages than in flat sedentary animals. The mantle margin of Doridoeides with the hepatic ramifications within it corresponds to the cerata of an Æolid, but is less extensive in surface, less mobile and therefore less efficacious for aerating.

As mentioned above, Doridoeides approaches most nearly to Pleuroleura of known nudibranchs, but the resemblances though important are somewhat general, and may be due to convergence. If a phylogenetic connection is accepted, the fact that both Pleuroleura, which is probably fossorial, and Doridoeides, which probably lives on

the leaves of seaweeds, are devoid of gills makes it likely that these gill-less forms are more ancient than Pleurophyllidia, and not retrograde.

Though Doridoeides is superficially not unlike Corambe (Doridella, Hypobranchiæa) it is not nearly allied to either the Corambidæ or the Phyllidiidæ. Both these families are holohepatic, and have a totally different arrangement of the mouth parts: branchial lamellæ situated beneath the mantle edge are found in all the genera comprised in them.

The heart and circulatory system of Doridoeides offer some points of interest. The heart lies somewhat to the right of a median line drawn longitudinally through the viscera, and the auricle adheres to the right wall of the pericardium. This may be a reminiscence of an earlier arrangement in which there was a ctenidium on the right hand side communicating with the auricle. The walls of the heart are thin, and in many sections the organ has an unsubstantial and shadowy appearance. The arteries also are thin, and hardly extend beyond the middle fifth of the body either backwards or forwards. They are developed most fully in the smaller specimens, and seem to atrophy as the animal grows. A similarly feeble development of the heart and circulatory system seems to occur in other gill-less nudibranchs. The Scaphopoda have neither gills nor heart. Kovalevsky[1] had some difficulty in seeing the heart in Pseudovermis and Hedyle, and could find it only in one species of the latter. In Tritoniella the heart lies to the right of the median line, and Bergh says of Pleuroleura ornata, "die aorta konnte nicht verforgt werden."[2] It would seem that in a gill-less mollusc the heart has no power of collecting purified blood and distributing it over the body, for the purification takes place all over the surface, not in a special organ. A strong pulsating machine and an extended

[1] 'Mémoires de l'Acad. Imp. des Sciences de St. Pétersburg,' vol. xii, Nos. 4 and 6.

[2] "Mal. Unters.," in Semper's 'Reisen.,' Heft vi, p. 284.

arterial system are, therefore, unnecessary. All that is required is sufficient movement to keep the blood from stagnating. But the extensive venous system is provided with valves which we have not noticed in other molluscs or seen described. They, presumably, serve to regulate and control the circulation.

EXPLANATION OF PLATES 15 AND 16,

Illustrating the paper by Sir Charles Eliot and Mr. T. J. Evans on "Doridoeides gardineri: a Doridiform Cladohepatic Nudibranch."

PLATE 15.

FIG. 1.—Doridoeides gardineri. *A.* dorsal, *B.* ventral view of whole animal. *a.* Mouth. *b.* Genital orifices. *c.* Anus. *d.* Accidental folds of foot. The specimen has been selected as showing the orifices, but in its natural shape the foot appears to be triangular, with a straight broad margin below the mouth.

FIG. 2.—Transverse section of dorsal body-wall.

FIG. 3.—Central nervous system of Doridoeides gardineri. *a.* Cerebro-pleural ganglia. *b.* Pedal ganglia. *c.* Eyes and olfactory ganglia. *d.* Buccal ganglia.

FIG. 4.—*a.* Jaw. *b.* Denticulate edge of ditto.

FIG. 5.—*a.* The radula seen from above. *b.* Side view of median tooth. *c.* Side view of first lateral. *d.* Side view of fourth lateral.

FIG. 6.—Alimentary system. *a.* Œsophagus. *b.* Anterior portion of stomach. *c.* Posterior do. *d.* Intestine. *e.* Right liver duct and branches. *f.* Posterior do. *g.* Left do.

PLATE 16.

FIG. 7.—*a.* Ventricle. *b.* Auricular ventricular valve. *c.* Auricle. *d.* Auricular extension. *e.* Aorta. *f.* Renal pericardial opening. *g.* Line of adhesion of auricle to ventricle.

FIG. 8.—Vein showing valvular construction.

FIG. 9.—Reproductive system. *a.* Hermaphroditic gland. *b.* Duct of do. *c.* Ampulla of do. *d.* Vas deferens. *e.* Penis. *f.* Bifurcation between male

and female branches. *g*. Spermatotheca. *h*. Vagina. *i*. Opening of mucus gland. *l*. Albumen gland. *m*. Mucus gland.

Fig. 10.—Section of hermaphrodite gland and adjoining parts. *a*. Male loculus containing spermatozoa in various stages of development. *b*. Female loculi. *c*. Do. showing communication with central male loculus. *d*. Dorso-ventral muscle bands passing through the hermaphrodite gland. *e*. Branches of the kidney. *f*. Section of main posterior liver duct.

Fig. 11.—Longitudinal section of whole animal, slightly to left of median line. *a*. Mouth. *b*. Radula sac. *c*. Anterior part of stomach. *d*. Posterior do. *e*. Spermatotheca. *f*. Ampulla of hermaphroditic gland. *g*. Duct of hermaphroditic gland. *h*. Hermaphroditic gland. *i*. Hepatic diverticula. *k*. Kidney. *l*. Blood spaces. *m*. Mucus gland. *n*. Salivary gland.

Fig. 12.—Transverse section of whole animal. Lettering as in fig. 11.

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I. Introductory.

In former memoirs (1896, 1898, 1905 [1]) I have proposed to divide the Ascons, or Calcarea Homocœla, into two families, Clathrinidæ and Leucosoleniidæ, differing

from one another in all those characters which, in my opinion, are of fundamental importance for the classification of Calcareous Sponges. I believe that the two families in question represent the most deeply-rooted phylogenetic divergence in the Calcarea Homocœla, and are two natural systematic groups, one of which—namely, the Leucosoleniidæ—approaches far nearer to the ordinary Heterocœla, such as Sycon, Grantia, Leucandra, etc., than does the other. The genus Leucosolenia, Bowerbank, in the sense in which the name is employed by me (1905 [2]), constitutes the principal, if not the only, genus in the family Leucosoleniidæ, while the genus Clathrina occupies a similar position in the Clathrinidæ.

Having in a former memoir (1898) described the origin and growth of the triradiate and quadriradiate spicules in various species of Clathrina, it seemed to me important to supplement the results then obtained by a study of spicule-formation in Leucosolenia. The observations put forward here have been carried on, at rather long intervals and with many interruptions, over several years, and some of my figures are now more than eight years old. I may be allowed to mention this in order to explain the relation of my investigations on calcareous sponge-spicules to those already published by my friend and former pupil, Mr. Woodland. After I had observed the principal facts in the development of the spicules of Leucosoleniidæ I suggested to Mr. Woodland that he should study the spicule-formation in the Heterocœla; he proceeded to do so in Sycon, and found a mode of origin for the spicules in this sponge perfectly similar to what I had found in Leucosolenia. I had intended to publish my observations a long time ago, but various obstacles and preoccupations prevented my doing so, and in the meantime Mr. Woodland's studies have been published (1905). It is in no way with the view of detracting from the value of Mr. Woodland's researches, which he has extended over a wide field, but simply in order to protect myself against a possible charge of plagiarising from his results that I point out here (as Mr.

Woodland has also done) that my figures and investigations on calcareous spicules antedate his, though published later.[1]

In my former memoir (1898) I went fully into the literature of spicule-formation in sponges and discussed the various statements of fact or theory made by those who have written upon this subject.[2] I may refrain from discussing further the historical side of the subject, since most of the papers dealing with the spicules of sponges that have been published during the past few years will be found quoted by me, with brief abstracts, in the 'Zoological Record' (1900—1903). I shall therefore only refer to the statements of other authors as occasion arises. In one point the published statements upon spicule-formation led me astray in my former memoir. Having then had no opportunity of investigating primary monaxons in the Clathrinidæ, I took for granted the correctness of previous statements upon the formation of monaxon spicules in sponges with regard to one point, namely, that each such spicule developed and grew in a single cell. This alleged mode of development was an obstacle to my comparison of the triradiate spicule to a system of three monaxons joined together, since each ray of a triradiate is formed from the first by two cells. When I came to investigate the monaxons of Leucosolenia, however, I found that each spicule of this class, whatever its size or form, arose invariably in two cells, exactly as does a single ray of a triradiate system. This fact had previously been observed by Bidder (1898, p. 62, foot-note) in the case of the hair-like spicules of Grantia compressa, and was regarded by him as specially characteristic of this type of monaxon; but from the observations of Woodland and myself, it would appear to be uni-

[1] I take this opportunity of remarking that I regard Woodland's "Studies in Spicule Formation" as constituting a most valuable contribution to our knowledge, so far as matters of fact and observation are concerned; but that in matters of theory I am often unable to see eye-to-eye with him. I may be obliged in the course of this memoir to controvert his speculative opinions.

[2] Maas (1900 [3], p. 555) is very charitable to me in attributing to design, and not to inadvertence, the fact that in my memoir (1898) I overlooked the statements of Deszö on spicule-formation in Tethya.

versally true, at least for the primary monaxons of Calcarea, that each such spicule is produced by two formative cells. This removes the only difficulty in the way of deriving the triradiate systems from a fusion of originally separate monaxons.

The species of Leucosolenia studied by me are L. complicata and L. variabilis. I have elsewhere (1905 [**2**]) given a detailed description of the characters, and especially of the spiculation of these two species. The material was obtained from Plymouth, and I have to thank the authorities of the Marine Biological Association for the trouble they have taken in collecting and preserving these sponges according to my directions. The technique used by me was the same as in the case of Clathrina, namely, preservation fresh from the sea in ½ per cent. osmic acid, staining with picrocarmine (either Ranvier's or Weigert's picrocarmine, supplied by Grübler and Co.), and examination in neutral glycerine or Canada balsam. This method is excellent for all protoplasmic structures, and does not corrode the spicules in the least; but it does not show details of nuclear structure, though the nuclei as a whole are well shown and clearly differentiated from the cell-body. For nuclear structures acid fixatives are indispensable, but then, of course, spicular structures are destroyed.

I have studied the stages of spicule-formation principally in surface-views of the thin wall of the sponge laid out flat, and rely upon sections only for confirming and supplementing the results so obtained. But I have abandoned the method which I formerly practised of brushing the collar-cells away with a paint-brush, as I now believe this procedure to be a dangerous source of error. The young stages of the spicules are found so close to the gastral epithelium (see especially Pl. 18, figs. 50—52, 54, 55, etc.) that I am now convinced that brushing may remove spicule-forming cells as well as collar-cells, and that some of my former figures (1898, Pl. 38, figs. 9, 12) are, in consequence, erroneous and incomplete, as I shall have occasion to point out further below. I

now rely on the region of the oscular rim, where the collar-cells are entirely wanting. By carefully cutting off the sponge-wall in this region and laying it out flat without any further treatment, stages of spicule-formation can be found and drawn, and thus any possible error arising from manipulation is entirely eliminated.

In Leucosolenia the dermal epithelium covering both aspects of the oscular rim is comparatively free from the coarse granulations found in Clathrina, a fact which makes the former genus a particularly favourable object for demonstrating the stages of spicule-formation, even to classes of students. It is interesting to find that in these days of advanced technique, there are still histological facts to be discovered by methods which might have been feasible to Lieberkühn or Max Schulze.

The investigations set forth in the following pages are not quite so complete in some points as I could have wished. Since, however, I now hold an appointment in which it is accounted to me for unrighteousness to look at a sponge (except for domestic purposes), I am obliged to put forward my results as they are without spending time upon further investigations.

II. The Monaxon Spicules.

The formation of the monaxon spicules of Leucosolenia takes place in a manner essentially uniform, in all cases observed by me, whatever the shape or size of the spicule. The process may therefore be described in general terms, and the variations observed in particular cases will be briefly noted afterwards. As a preliminary it should be pointed out that the full-grown monaxon spicules of Leucosolenia (the statement can, perhaps, be extended to the primary monaxons of all Calcarea) are placed invariably with one end, which may be called proximal, imbedded in the body-wall, while the opposite, or distal extremity, projects freely into the water. Haeckel (1872, vol. i) states that the

monaxons have their long axis parallel to the canal, in the wall of which they lie (l. c., p. 297), and are placed "in a meridian of the direction of the current" (l. c., p. 298). A glance at any Leucosolenia is sufficient to dispel this illusion; in all parts of the sponge the monaxons are found pointing in every possible direction (compare Fig. 108, Pl. 21); only at the oscular rim is a slight tendency to regularity noticeable.

The first sign of the formation of a monaxon spicule is the division of the nucleus of a dermal epithelial cell, followed by incomplete division of the cell-body (fig. 1—3, 41, 73—75). This division of the mother-cell, as it may be termed, marks out the two formative cells, which are not, as a rule, completely separated from one another in early stages, but later become distinct and largely independent of one another. A further point to note is that the formative cells retain a connection with the dermal epithelium from which they are derived (figs. 43—48; especially fig. 45). This point is of importance, since Woodland (1907) has based theoretical deductions upon the assumption that the scleroblasts have "lost connection with the rest of the organism" (l. c., p. 60). He even speaks of the scleroblasts as "wandering cells," meaning thereby, as I understand him, not that they belong to the class of amœbocytes (i. e. archæocytes), but that they are completely independent of other histocytes. It is always more easy to overlook than to discover connections between cells, and, therefore, positive observations are of more value than negative ones in their bearing on questions of this kind; but, in any case, I should doubt if scleroblasts, or skeletal tissue generally, could be supposed to be more independent than any other class of tissue-cells, and I consider the term wandering cells misleading as applied to the scleroblasts, and likely to produce a mistaken theoretical bias.[1]

[1] Woodland admits a connection between the scleroblasts and the dermal epithelium in some cases (1905, p. 243, and Pl. 15, figs. 45—49), but considers that in other cases no such connection is retained (l. c., fig. 48).

Each of the two formative cells plays a peculiar and characteristic rôle in the production of the spicule; one places itself more proximally, that is to say, deeper in the body-wall, and migrates towards the gastral surface; the other remains in a more distal, that is to say, superficial position, and is found in, or near to, the dermal epithelium. The proximal cell is conspicuous as a rule, for its more irregular form and amœboid appearance. As it travels inwards it forms the shaft of the spicule, and its chief function appears to be to determine the length and direction or curvature of the spicule. Like the apical formative cells of the rays of the triradiates, its influence is apparently chiefly directive; hence it will be briefly referred to in the sequel as the founder. On the other hand, the more superficially placed formative cell is more compact as a rule, and is concerned chiefly with building up the spicule to its full thickness. Like the basal formative cells of the triradiates, its function is essentially secretive; hence it will be termed for short the thickener.

Founder and thickener are at first scarcely distinct from one another, and the first sign of the spicule is seen between the two nuclei, but chiefly in the sphere of influence of the thickener (figs. 3—5, 9, 42, 45, 48, 76, 77). The earliest part of the spicule to appear is the distal extremity, the end,

A further point which Woodland has raised, and upon which it is more difficult to obtain decisive evidence, is whether or not the monaxons have in all cases a single mother-cell. He is of opinion that in some cases two distinct epithelial cells co-operate, and furnish the two formative cells. His observations could, however, be interpreted as meaning simply that in some cases the division of the primary mother-cell is complete, in other cases incomplete; and since the two cells become perfectly distinct later on, as Woodland himself has shown, the former procedure is more likely to be the primitive one. For my part I am inclined to regard the monaxons as uniform in origin and to refer them in all cases to a single mother-cell, but from the nature of the case it is almost impossible to give a decisive judgment on this point; nor do I consider it a fair argument to attempt to prejudice the point, as one might be tempted to do, by the analogy of the formation of the rays of the triradiates.

that is to say, which ultimately projects freely from the sponge-wall into the surrounding water. In most cases the free extremity of a monaxon spicule is marked by the possession of a so-called lance-head, a characteristic feature which is seen, when carefully examined, to be in reality a double bend in the shaft of the spicule, comparable rather to a bayonet than to a spear-head. The thickener remains attached to the head until this part has attained its full thickness and dimensions, but the founder travels away from the head, and lays the foundation of the shaft as a slender rod, which ends at first in the sharpest of points, and is far thinner than it becomes later (figs. 5, 6, 10—12, 43, 78). When the head of the spicule is fully formed, the thickener migrates slowly along the shaft towards the proximal extremity to which the founder is attached, and as it travels down it builds up the shaft to its full, definitive thickness (figs. 7, 13—16, 79—81). No further increase in size is possible for that part of the spicule beyond which the thickener has passed in its course towards the proximal end. Thus the function of the thickener is to finish what the founder has begun. In many cases the activity of the thickener is indicated by a distinct rim, marking off the distal portion of the shaft, formed to its full thickness, from the proximal slender portion laid down by the founder alone (figs. 13, 14*r*, 79, 80, 87). As the distal extremity of the spicule is completely formed it begins to project from the dermal surface of the sponge, being pushed outwards from the body-wall. How this extrusion of the spicule is effected is not quite clear, but it is perhaps due to the secretive activity of the founder-cell. The migration inwards (i. e. gastral-wards) of the founder in the early stages of the growth of the spicule soon brings it in contact with the bases of the collar-cells (see figs. 43—45, 71*a*, 71*b*). Beyond this point it cannot go, and the subsequent growth in length of the spicule is not really due to any further migration of the founder in a proximal direction, but to a pushing out of the spicule in a distal direction. Though the founder and thickener appear

to migrate along the shaft in a proximal direction, they are, in reality, practically stationary, while the spicule moves outwards. Sections show that the thickener retains its connection with the dermal epithelium, and forms a constituent cell of this layer (figs. 42, 43, 45—48, 57). Very noteworthy is the frequent presence in the founder-cell of a distinct, clear space in the protoplasmic body, continuing the shaft of the spicule, and appearing like a mould in which the secretion of the growing shaft is laid down (figs. 5—7, 13, 15, 79—83, 85, 86). When the preparation has been very slightly corroded by the mounting medium or some other cause, the proximal end of the shaft often appears hollow, containing a cavity or canal continuous with the clear space in the protoplasm of the founder-cell (figs. 83—86). This appearance, perhaps, indicates that the first secretion produced is of organic material which becomes subsequently impregnated with calcite to form the spicules, an interpretation further borne out by the observation that in some cases, in preparations in which there was not the slightest evidence of corrosion, the very young spicule appears first as a clear space within the protoplasm of the two formative cells, and that the calcite, when it makes its appearance, does not at once completely fill this space (figs. 3—5). In this connection I may draw attention to the very interesting results of Maas (1904 [1], [2], 1906), who has found that sponges reared in water deprived of $CaCO_3$ produce organic sclerites, not containing calcite.

When the spicule is nearly fully formed the founder ceases its activity. As the thickener, however, continues to secrete and to pass towards the proximal end of the shaft, the two formative cells are brought close together (figs. 7, 8, 17, 18, 44, 57, 71A, 82, 83, 85—88). When the spicule is fully formed, both cells leave it altogether. According to Woodland (1905, p. 242) the founder leaves it first (compare my fig. 8) and the thickener later; a fact which proves clearly the complete distinctness of the two formative cells in the later, if not in the earlier, stages of spicule-formation. The

thickener rounds off the proximal end to some extent and completes it. Full-grown spicules can always be distinguished from those not quite completed by the condition of the proximal end (compare figs. 87, 88, and 89). With a little practice the eye picks out the unfinished spicules rapidly in the preparation, and more careful inspection reveals at once the two formative cells on the shaft. The abandonment of the spicule by the formative cells is practically the only point in which the development of the monaxon spicules differs from the rays of the triradiates, on which one cell, at least, namely the thickener, is retained.[1] The difference is perhaps related to the fact that the triradiate systems are entirely imbedded[2] in the body, while one end of the monaxons always projects freely. The monaxons are probably being continually shed, or, it may be, drawn out of the sponge, like the hairs of many caterpillars. Hence the cells leave them, and new monaxons are continually being formed. The triradiate systems, on the other hand, are more permanent skeletal elements, their chief function being support, and not defence or protection, and in consequence they retain their formative cells.

I pass on now to describe briefly some of the peculiarities of the formation of monaxon spicules in particular cases.

(1) Leucosolenia complicata. In this sponge there are two sharply distinct types of monaxon spicules (*a*) small, straight, slender forms, and (*b*) large, curved, stout forms. No transitions are found between these two types (Minchin, 1905 [2]).

(*a*) The slender monaxons are very abundant, as a rule, and their early stages are easily found, especially in the oscular rim. Sections show that in this region they may be

[1] Compare p. 319 below.

[2] Woodland (1905, p. 237) states that in many Ascons the triradiates protrude through the thin body-wall, "so resembling monaxons." I can only say that no such Ascons are known to me. I may point our further that the development shows clearly that the apices of the rays of the triradiates are comparable to the proximal ends of the monaxons, not to the distal projecting ends.

formed in the interior of the sponge wall, projecting into the gastral cavity (figs. 46, 57, 68), in which case they arise from the dermal epithelium lining the gastral wall of the oscular rim in exactly the same way as they are formed on the outer surface of the sponge. The fact that the inturned dermal epithelium, which is for the most part destined to give rise to porocytes, can also secrete monaxons, is an important point in considering the origin of the gastral rays of the quadriradiates.

The slender monaxons of L. complicata are remarkable in that the lance-head is either rudimentary (fig. 7) or absent (fig. 8). The stages in the formation of these spicules are shown in surface views in figs. 1—8, and in sections in figs. 42—47 and 57.

The most noteworthy points in this development are, first, the great distance between the two formative cells before the spicule appears; and secondly, the clear space, which has been described above, which precedes the formation of the spicule (figs. 1—5). In places where the body-wall is thickened, for instance, at or near the point of attachment of the sponge, the spicules may be formed very far in from the surface (fig. 45).

(*b*) The stout monaxons are fairly abundant, but their earliest stages are, for some reason, difficult to find; perhaps because they grow with great rapidity. As a rule they have a very distinct lance-head, and figs. 10—15 show, without need of further explanation, how the lance-head is formed by the thickener and then gradually abandoned by it. Occasionally, however, the lance-head is completely absent (fig. 16). The great size of these spicules makes it very difficult, in fact, practically impossible, to obtain perfect stages of their growth in sections. Some are shown in figs. 48, 71 *a*, 71 *b*.

When I commenced my investigations on these spicules I made use of the method, which I now condemn, of brushing off the gastral collared epithelium. In such preparations I found many young spicules, similar to figs. 10 and 11, but

with only one cell on the spicule, this cell being situated on the lance-head in the position of the thickener. At first I regarded this state of things as normal, but I am now convinced that it arose simply by the brush having removed the founder, which is often situated very close to, or even among, the collar-cells. (Compare Figs. 48 and 71 *b*.)

(2) Leucosolenia variabilis. In this sponge the ordinary monaxons vary greatly in size but agree in their characters, and form one class in which every gradation can be found between the two extremes of size. They have distinct lance-heads and long, curved shafts. Two other types of monaxons are also found, but they are very scarce, and I have not found any stages of their development.

The ordinary monaxons may conveniently be grouped into small (fig. 83), medium (fig. 86), and large (fig. 89), but these three sizes are connected by every possible gradation, and must not be regarded as indicating distinct subdivisions. There is nothing to note about their mode of development which has not been said already, but some conclusions of importance from a systematic point of view may be drawn from the stages of growth. It is sufficiently obvious from the figures that small monaxons like fig. 83 are by no means to be regarded as young stages of large monaxons like figs. 88, 89. On the contrary the future size of the spicule is determined at a very early stage—that is to say, as soon as the lance-head is formed (compare figs. 78 and 84). Further, the study of the development affords a means of distinguishing the spicules that have or have not attained their full growth, as already pointed out. In describing the spiculation of a sponge for purposes of specific identification it is most important that the description should apply only to fully-formed spicules, and not to those in which the growth is not complete.

The mode of development of the monaxon spicules that I have described in the foregoing is one that I have found in all the monaxons of Leucosolenia that I have studied, and Woodland, whose preparations I have looked through, has

found a similar course of events in the formation of the monaxons of Sycon. Maas, however, has given a description of the formation of the monaxons of Sycandra setosa, which is at variance with our results (1900 [2]). He finds the small monaxons developing each in a single cell, but the larger monaxons, on the other hand, being built up by numerous cells. It may be pointed out, to begin with, that Maas has described these appearances from sections, which is an unsafe proceeding. I should interpret his fig. 23 on Pl. 11, as representing one of the two cells present, probably the thickener, on each of the three spicules depicted, the other cell having been cut away or overlooked. Again, his fig. 25, intended to represent a large monaxon with many cells on it, is not convincing to me, as it is not possible with the magnification given (300) to determine the relation of the cells to the spicule. The drawing shows six rounded cells on or over the spicule, none of which look at all like scleroblasts. I may remark that the monaxon in question is very much smaller than the large monaxons of L. complicata (compare my figures of the spicules at the same magnification [1905 (2)]), and that in this case, as already described, I have found never more than two cells present on the spicule. I regret, therefore, that I am unable to accept my friend's figures as evidence for the correctness of his statements, which appear to me to require revision.

A monaxon spicule which develops, like a single ray of a triradiate, from two formative cells derived by division of a single mother-cell, is a spicule which I should regard as a primary monaxon. So far as the observations of Woodland and myself extend, the monaxon spicules of Leucosolenia and Sycon are all primary. There is no reason why a monaxon spicule should not arise secondarily, by modification of a triradiate, that is to say, by loss of one ray and placing the two remaining in a line with the other. I have elsewhere (1905 [3]) given reasons for regarding the huge monaxons of Clathrina contorta as secondary in nature, and I feel convinced that the same applies to the elbowed monaxons in

the stalk of C. lacunosa, and probably to many other cases also. In all the species of Clathrinidæ in which I have been able to follow the development, the first spicules formed after the metamorphosis are triradiates, and monaxons, if present, appear much later. On this account I am inclined to doubt if primary monaxons occur at all in Clathrinidæ. In the few cases in which I have found developing monaxons in species of Clathrina, there were several formative cells attached to them, at least four, and not two only, as in Leucosolenia. I had greatly hoped to have been able to study the development of monaxon spicules in Clathrinidæ, the only thing required to complete our knowledge of the formation of spicules in Homocœla. I must, however, leave this task to others, and can only say at present that there is some evidence for believing the monaxons of Clathrinidæ to be of secondary nature. I may add that there is no reason why secondary monaxons should not occur in Leucosoleniidæ, but there is at present no evidence that they do. Maas's statements raise a presumption in favour of the occurrence of secondary monaxons in Sycons, but his single observation does not convince me on this point.[1]

According to Maas (1900 [**1**], p. 44; [**2**] p. 225) the small monaxons of Sycandra setosa grow at first slowly within a cell, until they project from the cell and from the tissue, and then a sudden, rapid growth of the spicule takes place, like the shooting out of a crystal ("Krystallartiges Anschiessen"). If I have understood the author's meaning rightly, he considers the portion of the spicule imbedded in the tissue as the older part, and the portion projecting into the water as a more recent formation. My observations lead, however, to an exactly opposite conclusion. The development of monaxon spicules possessing a lance-head shows clearly that the distal extremity of the spicule is the part first formed, and the proximal portion is the last to be secreted by the scleroblast.

[1] I consider it possible that the bayonet-like monaxons described by me (1905 [**2**]) in Leucosolenia variabilis (l. c., text-figs. 95, 16 *g*, 17 *a*, *b*) and L. botryoides (l. c., text-figs. 98, 25 *i*, *j*) might be reduced triradiates.

III. The Triradiate Systems.

One of the distinctive characters of the genus Leucosolenia, as defined by me in a former memoir (1905 [**2**]), is seen in the form of the triradiate systems. In Clathrina these spicules are typically equiangular, and only very exceptionally depart from this type, equiangular triradiates being in all cases present and constituting the principal skeleton. In Leucosolenia, on the other hand, the triradiates exhibit typically a bilaterally symmetrical pattern; they have a unpaired straight ray and two paired, usually curved rays, and corresponding to these differences in the rays, there is an unpaired angle greater than 120°, and two paired angles each less than 120°. Only exceptionally, and as one of many variations, are the three rays equally developed, or the three angles each indistinguishable from 120°. The two species in which I have studied the spicule-formation are further distinguished from one another by the characters of their triradiates. In Leucosolenia complicata the unpaired ray of the triradiate is almost invariably longer than the paired rays; in L. variabilis the unpaired ray is constantly the shorter of the three. Moreover, in L. variabilis the unpaired angle is much more obtuse than in L. complicata, and often approaches 180°, while in L. complicata it more nearly approximates to 120°.

If an ordinary museum-specimen, preserved in spirit, of L. complicata be taken, and a piece of the thin body-wall cut out, mounted without further treatment, and examined microscopically, with moderately high magnification, it is easy to see many stages of the growth of the triradiates, especially if the preparation be examined by polarized light with crossed prisms, when the spicules stand out brilliantly illuminated on a dark background. It is then seen that the stages of growth are as follows:—first a small piece of calcite in the form of an isosceles triangle, in which the base is about one-third the length of the sides; this represents the

unpaired ray. Next, from the base of the triangle two tiny rays grow out, which become larger and take shape as the two lateral rays of the spicule, growing rapidly, and so reducing the disproportion between the paired and unpaired rays, which is at first so marked. From the comparison of a series of young stages in the growth of the spicules the impression gained is that the triradiate system is laid down first as a monaxon spicule representing the unpaired ray, and that this monaxon spicule branches at one end, thus giving rise to the two paired rays. This interpretation of the observations seemed to me so obvious that, until I had studied the behaviour of the formative cells, I regarded the spicules of Leucosolenia as arising by secondary branching of a monaxon, and, therefore, of a fundamentally different type from those of Clathrina, which represent a system of three monaxons joined together. As soon as I had studied the histological details of spicule-formation, however, I found this interpretation to be quite erroneous, since the triradiate systems of Leucosolenia develop in just the same way as those of Clathrina, namely, from a sextett of cells, two of which give rise to each ray of the spicule. The rapid growth of the unpaired ray of L. complicata is merely an interesting case of precocious formation which is prophetic, so to speak, of the great size to which this ray ultimately reaches (figs. 21—29). If one examines the development of the triradiate systems of L. variabilis, in which the unpaired ray is the shortest of the three, it is found at once that there is no such precocious development of any one of the three rays.[1]

The "sextetts" of formative cells from which the spicules arise are found without difficulty in surface views or sections (figs. 19, 20, 51, 54, 55, 71, 90, 91). Though I do not doubt that each sextett arises, as in Clathrina, by division of each cell of a "trio," it is more difficult to be sure of this in Leucosolenia. I have not found any distinct trios, as I did in Clathrina, and, except for the argument by analogy

[1] Woodland has recorded analogous differences in the development of the triradiates of Sycon ciliatum and S. coronatum (1905, p. 245).

furnished by the development of the monaxons, it would be quite feasible to regard each sextett as having arisen by the immigration of six cells independently from the dermal layer. The cells of the sextetts are very irregularly disposed, and it is difficult, often impossible, to pair them off, so to speak. Frequently a seventh cell, slightly more granular than the remaining six, is found, forming a septett; in these cases the spicule is destined to be a quadriradiate, and the seventh cell is a gastral actinoblast, the mother-cell of the gastral ray. I have never observed, however, the gastral actinoblast to be present until the triradiate system has begun to be formed and has reached a certain size.

Although I have examined carefully some hundreds of young stages in the formation of calcareous triradiate systems, both in my preparations and in those of Woodland, I have never seen anything but the two conditions I have just described, that is to say, either six or seven cells. Urban (1905, Pl. 6, fig. 34) has figured, however, a triradiate system with more than six cells upon it. I am inclined to think that this author has not clearly distinguished between formative cells adherent to the spicule, and cells of the overlying dermal epithelium. It is very easy to confuse the two. More difficult for me to understand are the statements of Maas (1900 [2]), who claims to have found the quadriradiates of Sycandra setosa arising each in a single cell (l. c., Pl. 11, fig. 24, i—iii). This statement is so directly at variance with all my experience on the subject that I am unable to support my friend's statements. I think the discrepancy in our observations may be explained in the following way. In the septett of cells covering a young quadriradiate the gastral actinoblast is far more granular than the formative cells of the basal system, and this difference is accentuated if alcohol is used as the preservative (which was Maas's method) instead of osmic acid (which I always employ). Looking down upon the septett from the gastral aspect, the gastral actinoblast would be obvious, but the cells of the underlying sextett might easily be overlooked if

they were faintly stained. One would then get the impression that the entire spicule was the product of one cell, namely the gastral actinoblast. I may point out that so careful an observer as Metschnikoff made, many years ago, a similar misinterpretation of the sextett surrounding the young triradiates.

It is never safe to be dogmatic about things one has not seen, but I venture, nevertheless, to express my belief that it will be found to be universally true that the triradiates of calcareous sponges develop from six cells,[1] the quadriradiates from seven.

As regards the subsequent development of the triradiate systems, the process of events is quite similar to what I formerly described in Clathrina, as may be seen from my illustrations. The first deposits of calcareous matter are very irregular (figs. 19—21), but soon take definite shape as a symmetrical spicule, and, concomitantly, the cells of the sextett sort themselves out into three pairs of formative cells, two attached to each ray in a definite and uniform manner. One formative cell, placed more towards the gastral aspect of the body-wall, behaves in all respects like the "founder" of the monaxon spicule; the other, placed more on the dermal side, is the "thickener" (figs. 24—28, 52, 58, 92—95). The founder is to be sought for at the tip of the ray, the thickener at the base; hence these two cells were distinguished by me in a former memoir as the apical and basal formative cells, respectively. As development proceeds, there comes a period when the founder is no longer to be observed, having wandered off from the ray. It is with regard to this point that I wish to correct some of my former statements. In Clathrina I figured a quite young spicule, in which the rays were far short of their full length, without any founder-cells attached to the extremities of two of the rays (1898, pl. 38, fig. 9). I am now convinced that this condition was simply due to the founders having been brushed off, since they are

[1] That is to say, in the first instance from three mother-cells, each of which divides into two, thus giving rise to the sextett.

placed very close to the gastral epithelium (figs. 50, 52). Examination of preparations not manipulated in any way shows that the founder, as in the case of the monaxons, does not leave the ray until it has attained its full length (figs. 29, 97, 99). In C. clathrus, as I showed formerly, the founder does not leave the ray at all and is thus responsible for the clubbed ends of the rays which are distinctive of this species.

As soon as the ray has grown to its full length, however, the founder disappears. The thickener has, meanwhile, migrated slowly outwards from the base of the ray, depositing lime and finishing off the ray as it passes along. Shortly before the disappearance of the founder, the thickener is found close to it just as in the monaxon spicules (compare figs. 97 and 99 with figs. 7, 8, 17, 18, 57, 71*a*, 82, 83, 85—88). In the fully-formed triradiate each ray bears the thickener at the extreme tip, generally in the form of a compact cell, but sometimes remarkable for sending out slender processes in various directions (figs. 97, 98). The thickener does not quit the ray, but can always be found attached to the tip of it. Woodland, however, states (1905, p. 145) that in Sycons both formative cells quit the rays of the triradiates. I can only say that in all the Ascons I have examined, I have never failed to find at least one cell on the apex of each ray of the full-grown triradiate systems.

IV. The Gastral Rays.

In the genus Leucosolenia the gastral rays of the quadriradiates are always curved and thorn-like, the apex pointing, typically, towards the osculum, i. e., in the direction of the water-current.

In the formation of the gastral rays, I found a curious difference between those formed in the oscular rim, above the level reached by the gastral epithelium of collar-cells, and those formed throughout the gastral cavity generally where it is lined by collar-cells. I will deal with the latter first. As

I have already stated, the gastral ray owes its origin to a granular cell which joins itself to the six formative cells of the triradiate system to form a septett. In Clathrina I was able to show that this cell, the gastral actinoblast, arose from a porocyte, or from one of the epithelial cells, lining the interior of the gastral rim, from which porocytes arise. In Leucosolenia the characters of the gastral actinoblasts leave no doubt in my mind that they have an origin similar to those of Clathrina, but I am not able to bring forward such definite proof of this statement. This is one of the points in which I had hoped to have completed my observations. I may draw attention, however, to the section figured in fig. 54, in which the cell (*p. c.*) could be interpreted as a gastral actinoblast in the act of migration from the neighbouring pore.

In L. complicata the nucleus of the gastral actinoblast divides sooner or later into two. Sometimes the division takes place very early (fig. 24).

There is little to note with regard to the growth of the gastral ray itself; it is sufficient to refer to the illustrations given (figs. 24—28, 60—70). I may draw attention, however, to the fact that the gastral ray of L. complicata arises far behind the junction of the three basal rays (figs. 24—26, 28, 52). This point is noticeable in the full-grown spicule, but much more so in the early stages of growth. Hence even quadriradiate spicules, at their first appearance, are utterly unlike the primitive tetraxon form, from which some authors derive all the spicules of calcareous sponges.

With regard to the cells found upon the gastral ray some remarkable peculiarities can be observed. One is the tendency of the cells to become increasingly granular, some of the granules being of large size and taking a pink stain with picrocarmine. Another point is the presence in many cases of additional cells of a peculiar kind on the full-grown gastral ray (figs. 64—67, *ex. c.*, 69); a point which I shall have to deal with under a separate heading.

As regards the gastral rays formed in the region of the

oscular rim above the limit of the collared gastral epithelium, I was unable to find actinoblasts upon them in sections, and it appeared to me as if the gastral rays in this region were formed directly by the activity of the dermal epithelium lining the interior of the oscular rim (figs. 56, 68). My observations, however, are not sufficiently extensive to prove this point to my satisfaction.

V. Derelict Spicules in Leucosolenia complicata.

In L. complicata I found frequently abnormal spicules which were evidently deformed triradiates or quadriradiates, and which were situated close to the dermal surface (figs. 30—36). Some had no cells attached to them, others had one or more cells, in one case (fig. 36) as many as four, but never the full number of six or seven found on normal spicules.

These peculiar bodies appear to me to be spicules which for some reason have become arrested in their development and abandoned by their formative cells at an early stage in their growth, and are about to be extruded from the sponge. In one case the appearance seen (fig. 35) suggests that the formative cells were in the act of migrating from the spicule. The peculiar forms of these spicules appear to be due to the fact that one or more of the formative cells may adhere to the spicule for some time, and continue to secrete calcite. Figs. 30 and 34 suggest this interpretation strongly. When all the cells have left them they become mere derelicts, which react as foreign bodies and are cast off.

If my interpretation of these peculiar bodies is correct, they afford a certain analogy with the formation of the large monaxons of certain Clathrinidæ. As I have stated above, I found four formative cells on the monaxons of an undetermined species of Clathrina; this observation, by comparison with the primary monaxons, indicates that the big, secondary monaxons of Clathrinidæ are biradiates, that is to say, are equivalent each to two rays of a triradiate, a view which I have maintained elsewhere (1905[3]) for those of

Clathrina contorta and C. lacunosa. I believe, however, that in an earlier stage the secondary monaxons would be found to be covered by six cells, of which two wander off, leaving the two remaining pairs to secrete the spicule. On this view the secondary monaxons would be comparable to a certain extent with the derelict spicules described above. I regret that I am not able to bring forward concrete evidence for these conclusions.

VI. Histological Notes; the Excretory Cells of Leucosolenia complicata.

The dermal epithelium of Leucosolenia may vary greatly in form, as Urban (1905, p. 53, pl. vi, figs. 42—62) has pointed out for another species. Some cells are of the conventional flat type (figs. 43, 54—57), from which every gradation can be found to others which have the nucleus placed deeper and are more flask-shaped (figs. 38, 47). Near the point of attachment the epithelium becomes of a pronounced columnar type (fig. 45). The species of Leucosolenia are very slightly, if at all, contractile, and contrast sharply in this respect with the Clathrinidæ. Hence, in agreement with Urban, I do not think that the flask-form can be explained in this genus as the result of contractility. I am not inclined, however, to regard the dermal epithelium as generally glandular, though it is very probably so in the region of the point of attachment. The clear protoplasm, free from coarse granules, of the general dermal epithelium does not suggest secretory cells. I regard the polymorphic nature of the dermal epithelium as due to the fact that it is practically a layer of amœboid cells, which are continually immigrating into the interior to form spicules and returning to the surface again. The power of concerted contractility possessed by the dermal epithelium of Clathrinidæ has apparently not been acquired by the Leucosoleniidæ, which in many respects present more primitive characters. In the collar-cells, for instance, the nucleus is terminal, as in

the larva, while in the Clathrinidæ the nucleus migrates into a position near the base of the cell.

Bidder (1898, p. 73) has pointed out that in Leucosoleniidæ the pore-cells, or, as he terms them, pylocytes, are not on the surface as in the Clathrinidæ, but at the bottom of short canals lined by the dermal epithelium. I am able to confirm this statement. Fig. 72 shows four porocytes and their relation to the dermal epithelium, drawn from the gastral aspect, the spicules and other cells being omitted. The structure of each pore and its enclosing cell is exactly as in Clathrina.

The amœbocytes in Leucoselenia are not so distinct from the other tissue-cells in appearance as they are in Clathrina, but can be distinguished by their larger nucleus (Figs. 49, 71, *amc.*). I found in Leucosolenia the same minute wandering cells, very abundant in some places, that I described formerly in Clathrina (fig. 71, *amc.*[1]).

In the great majority of works upon sponge-histology, it is customary to distinguish and to describe a class of connective-tissue cells, stellate or bipolar in form. Without wishing to make statements about other sponges, it is my firm opinion that no such cells occur in Ascons. In surface views of the body-wall it is easy to make out the various classes of cell-constituents, namely, dermal epithelial cells, porocytes, amœbocytes, scleroblasts attached to the spicules, and collar-cells, with the addition, in certain specimens, of generative cells. No separate connective-tissue cells are to be seen. In sections of the body-wall, however, the spicules are always more or less displaced (compare fig. 55), and their scleroblasts, left in position, give the impression of separate connective-tissue cells. If, however, the stellate cells, frequently described as being numerous, really occur, they should be visible in surface views, in which their absence is, in my opinion, a convincing proof that no such class of tissue-elements occur in these sponges.

I come now to a remarkable class of cells, which I discovered first when studying the gastral rays of the quadri-

radiates in sections. On many of the gastral rays I found, in addition to the two formative cells one or two conspicuous rounded cells (figs. 64—67, *ex. c.*), packed full of coarse granules which take the carmine-stain more or less distinctly. In young gastral rays these cells were absent, and they were frequently absent also in full-sized rays; when present there were usually two, but in sections one of them may, of course, be cut away. The most striking peculiarity of these cells is that their coarse granules are always arranged in a layer at the surface of the cell, and in many cases appear to be in the act of being cast off from the cell (fig. 64—67, 69). The appearances suggest strongly, in short, that the coarse granules represent some kind of excretory material which the cells are producing and throwing off.

After finding these cells on the gastral rays I searched for them in other places, and soon discovered that they were fairly common among the collar-cells, from which they could easily be distinguished by the characters of their protoplasm (figs. 43, 53, 57, *ex. c.*). I also found them frequently in the oscular rim, at or close to the uppermost limit of the gastral collared epithelium (figs. 56, 57). Their presence in the oscular rim shows that they must be derived from the inturned layer of dermal epithelium in this region, and that consequently they are cells of the same class as the porocytes, which they resemble in their characters. I take this opportunity of remarking that similar cells occur abundantly between the collar-cells of Ascandra falcata, projecting into the gastral cavity between the collar-cells, and that in Clathrina coriacea similar cells form the endogastral network described by me formerly (1899, p. 123, figs. 42 *c* and 46; 1900, p. 48).

As regards the existence of the excretory cells, as I believe them to be, upon the gastral rays, I was unable to decide to my own satisfaction whether they are formed where they are found, from the actinoblasts themselves, or whether they migrate on to the rays from their position between the collar-cells. If I am correct in regarding both the excretory

cells and the actinoblasts as derived from the porocyte-layer, then it is not impossible that the excretory cells might arise from the actinoblasts in some cases. Certain appearances suggest that the actinoblasts first become very granular and excretory in nature (fig. 59), and then give rise to separate excretory cells (fig. 69). On the other hand, I have frequently found the excretory cells near the base of young gastral rays, in a situation that suggests the possibility that they might migrate on to the rays (figs. 62, 63). I am obliged to leave this point undecided, and may remark that it would be better to study the question in surface-views of the inner aspect of the sponge-wall (as I had hoped to do) than in sections, since by the latter method it is never possible to be certain how much has been cut away in the preparation. Hence negative evidence is indecisive when derived from the study of sections.

With regard to the function of these cells, I may recall, in this connection the fact that Bidder (1892) regarded certain cells in Clathrina clathrus as excretory in function, basing this conclusion upon the reactions of these cells to stains. Bidder termed the cells in question Metschnikoff's cells. In my memoir on the Clathrinidæ (1898) I pointed out that the Metschnikoff's cells were contracted porocytes, and argued (l. c., p. 527) against the probability of the porocytes being excretory on the ground that any excretions produced by them would be carried into the sponge by the water-currents, whereas excretions are usually produced in situations where they are carried out of the body. I may point out, however, that the cells which I am describing in Leucosolenia, and which are also of the nature of porocytes, would be very favourably situated for exercising an excretory function, especially when they occur in the oscular rim or on the gastral rays of the quadriradiates. The cells of sponges, and especially of Ascons, are extremely generalised in function, and capable of exerting at different times activities which in other Metazoa are exerted by different cells, specialised each in a particular direction. Thus in

Clathrinidæ we see the contractile cells of the dermal epithelium migrating into the interior to become sclero-blasts, and probably returning again to the epithelium when this function is discharged. In the same way it is not difficult to imagine a cell which under certain circumstances becomes a pore-cell and under others becomes a gastral actinoblast, may under yet other conditions take on an excretory function. If Bidder's term "Metschnikoff's cells" is to be used at all, I should suggest that it be used for cells such as I have described here, namely, cells of excretory function derived from the porocyte-layer, that is to say, from that part of the dermal epithelium which lines the oscular rim and furnishes the pore-cells and the gastral actinoblasts. And I may point out, that at a certain period in the development, or at any time during life in many Clathrinidæ, when they are contracted to a certain point, the porocyte-layer forms the innermost lining of the gastral cavity, excluding even the collar-cells from it (1900, figs. 58, 4, and 42, F).

The collar-cells of Leucosolenia complicata are shown in figs. 37 and 53; in other figures they are represented in outline. They are more or less flask-shaped, with the oval or pear-shaped nucleus at the upper extremity, close below the collar. The flagellum can be traced down to the nucleus. The collar is long and cylindrical; its free rim is difficult to make out. About half-way up the collar shows a hoop-like thickening. The cytoplasm is clear and finely granular, occasionally with a few coarser refringent granules and usually distinctly vacuolated. In sections it is common to find a collar-cell cut in such a way that only the base is shown. I have drawn one cut in this way in fig. 56 (*c. c.*) in order to show the difference between it and an excretory cell (*ex. c.*).

A cytological study of the collar-cell, its nucleus, and the mode of division would be of great interest. Unfortunately the osmic-picrocarmine method used by me, though very good for cytoplasmic details, gives very poor results for nuclear

structure. From appearances such as are figured in fig. 37, and which are frequently met with, the collar-cells of Leucosolenia would appear to divide longitudinally.

VII. An Abnormal Triradiate.

Fig. 101 on Pl. 20 represents part of an abnormal triradiate system of Clathrina coriacea with the cells upon it. This drawing was made by me more than ten years ago, but I have always kept it back in the hopes of finding other abnormal forms, and devoting a special memoir to them. As will be seen, one ray of the triradiate has two branches symmetrically placed on each side of the main shaft, each branch with a cell similar in appearance to a "thickener" attached to it.

From a single observation of this kind it is difficult and unsafe to draw conclusions or to attempt to reconstruct the course of events. Did the founder and thickener go off in different directions? Or, after the founder had gone off, did the thickener divide into two and so produce two daughter rays? It is unfortunate that the spicule, having reached the limit, apparently, of its growth, does not furnish any answer to these queries. Two things may be noted, however; one is that the three principal rays of the spicule are unusually stout and large, indicating great secretive activity on the part of the formative cells; the other point to be noted is that the two branch-rays are set on to the main ray at the same regular angle of 120°, which characterises the junction of the three principal rays.

VIII. Some Observations of the Disposition of the Triradiate Systems in Leucosolenia lieberkühnii.

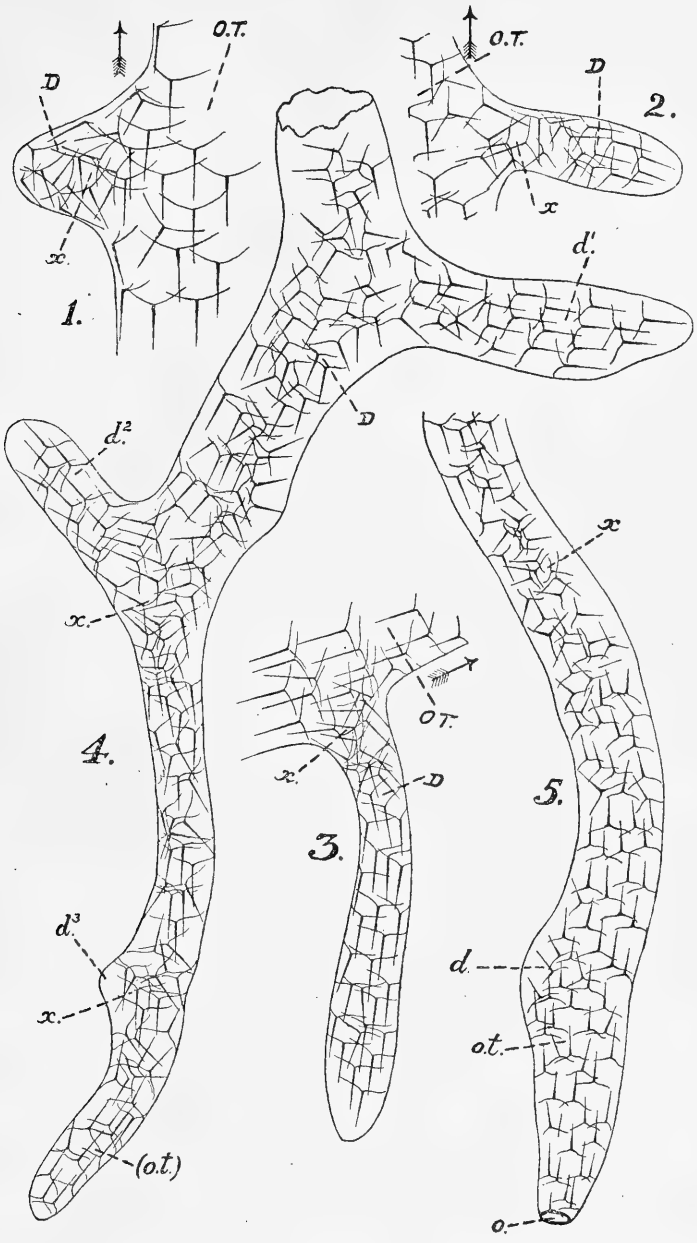
The observations to be recorded were made by me during my tenure of the Oxford Table in the Zoological Station at Naples during the year 1891–92. Leucosolenia lieberkühnii occurred then in the greatest abundance on the

bottom of a training ship moored permanently in the Porto Militare, growing amidst a thick crust of worm-tubes, Ascidians, sponges of various kinds, and other organisms. The colonies of Leucosolenia (Pl. 21, figs. 104—107) frequently attained a very large size, and were easily obtained from a boat by scraping the crust from the ship's bottom; during which occupation the too enthusiastic naturalist might sometimes receive on his head objects or liquids of a peculiar, and even offensive, nature from the port-holes of the vessel.

I made at that time a fairly complete study of the skeleton of this species of Leucosolenia, and found many errors in the published accounts of the spiculation. I will only refer at present to the peculiar slender monaxon spicules, shaped more or less like walking-sticks (Pl. 21, figs. 120—122), which I have found always present, though previously undescribed, in this species, not only in Neapolitan specimens but also in those from Banyuls, and other parts. I regard these spicules as most diagnostic of this species.

The triradiate systems (Pl. 21, figs. 109—114), both those with and those without an additional gastral ray, are, like those of other species of Leucosolenia, distinctly sagittal, having a straight unpaired "posterior" ray and two curved "lateral" rays. The "anterior" angle, between the paired rays, is greater than 120°; the lateral paired angles are less than 120°. The unpaired ray is sometimes shorter, more often longer, than the paired rays.

In the accompanying text-figure I have represented the disposition of the triradiates in different parts of the sponges. I wish to state that, as regards form, the individual triradiates are represented diagrammatically in the text-figure without attempting to reproduce the exact form of the spicules; on the other hand, as regards position of the spicules, the figure claims to be exact. My method was to mount pieces of the sponge unstained in Canada balsam, and to draw with the camera lucida all the triradiates seen in the body-wall on the upper side of the tubes. Owing to the thinness of the body-wall the refringent spicules can be made



Leucosolenia lieberkühnii.—1. Portion of the oscular tube with a young diverticulum. 2 and 3. The same, with older diverticula. 4. Diverticulum bearing secondary diverticula. 5. A newly formed (secondary) oscular tube. *O.T.* Primary oscular tube. *o.t.* Secondary oscular tube. (*o.t.*). Ditto, in which the osculum is not yet formed. *O.* Osculum. *D.* Primary diverticula. *d*, d^1, d^2, d^3. Secondary diverticula. *x*. Spots where the spicule-arrangement is confused. The arrows in 1, 2, and 3 point in the direction of the osculum.

out very easily in the transparent preparations. As the tubes are rounded, the spicules placed more towards the side are, of course, somewhat foreshortened. At first I used to draw the whole spicule, but I found it just as useful, and much less laborious, to draw only the straight posterior rays with the camera lucida, and to add the lateral rays freehand. It is for this reason that I state that the precise form of a given spicule is not to be considered as accurately represented in the figures. In fig. 108, Pl. 21, I have carefully drawn with a camera lucida the arrangement and form of the triradiate systems in one of the diverticula.

The sponge has erect oscular tubes, often of great size (Pl. 21, figs. 105, 107), from which grow out very numerous diverticula, ramifying in their turn (text-fig. 4). The diverticula are at first, and for a long time, blind at their ends (text-figs. 1—4), but become sooner or later perforated at their blind extremities to form oscula (text-fig. 5, *o.*), thus producing smaller oscular tubes which may be conveniently distinguished as secondary from the large erect primary oscular tubes.

Beginning the description with the primary oscular tube, a very uniform and regular arrangement of the triradiate systems is found (text-figs. 1, 2, and 3, *O. T.*; the arrows point in the direction of the oscular opening). Each triradiate is disposed with its straight unpaired ray pointing away from the osculum, and its two lateral rays extending in a direction as near to the horizontal as their form permits, that is to say, with their points slanting slightly upwards. In those systems which develop a fourth or gastral ray, and so become quadriradiates, it follows that the gastral ray curves forward to point towards the oscular opening, that is to say, that the gastral rays bend over and point in the direction of the water current, which flows up the oscular tube towards the terminal aperature. The suggestion that the gastral rays owe their curvature to the influence upon them of the water-current during development is an obvious one; we shall return to this point later.

From the oscular tubes arise, as already stated, numerous diverticula (*D.*), each an outgrowth of the body-wall pushed out, so to speak, like a glove-finger. Three stages are seen in text-figs. 1, 2, and 3. The triradiates formed in these diverticula take on from the first a different arrangement from those in the erect oscular tubes. At first the spicules are quite confused and irregular in arrangement (*x.*) As the diverticulum continues to grow, however, order emerges from chaos, and the triradiates are found uniformly disposed (text-figs. 2, 3, and Pl. 21, fig. 108). Each triradiate has its unpaired ray pointing towards the blind end of the diverticulum, but at the point of origin of each diverticulum from the oscular tube, there can be seen the region (*x.*) in which the chaotic and irregular arrangement of the triradiates is retained without any "righting" of the spicules having taken place. The arrangement of the triradiates in the diverticula of Leucosolenia was first pointed out by Bidder (1891, p. 627).

The diverticula are, of course, lined by collar-cells and perforated by pores, like the wall of the oscular tube, and it cannot be doubted that the water-current which flows in through the pores must pass along the diverticula in the direction from the apex to the base, finally debouching through the main aperture[1] into the oscular tube and joining the current in the latter. Hence it follows that the arrangement of the spicules in the diverticula is the same, in relation to the water-currents, as it is in the oscular tubes, with the exception of the basal region (*x.*). If the hypothesis that the gastral rays are bent by the current applies in the oscular tubes, it also applies in the diverticula.

The primary diverticula thrown out from the oscular tubes grow in length and produce secondary diverticula from their sides (text-fig. 4, *D*, d^1, d^2, d^3). The formation of a secon-

[1] Woodland in his comparison of the diverticulum to a "water cushion" (1905, p. 266) seems to have forgotten their aperture into the oscular tube. To my mind they can no more be compared to water-cushions than can the oscular tube itself.

dary diverticulum recapitulates exactly the events already described in the formation of a primary one; first, a chaotic arrangement of the triradiates (*x.*), then a quite regular disposition with the unpaired rays pointing to the blind apex.

When the diverticula have grown to a certain length, however, a remarkable change takes place in the arrangement of the spicules. In diverticula which still end blindly at the apex (text-fig. 4) it is seen that a short way from the apex the spicules become confused in arrangement and that from chaos an orderly arrangement again emerges (*o. t.*), in which, however, the disposition of the triradiates is exactly the reverse of what previously obtained; the unpaired ray points away from the blind apex of the diverticulum instead of towards it. As it cannot be supposed that the direction of the water-currents is changed so long as the diverticula end blindly, it follows that the gastral rays now point against the current, instead of bending with it.

The significance of the reversed arrangement of the triradiate becomes obvious when it is found that in other diverticula of about the same size or larger (text-fig. 5) the blind extremity becomes perforated by an aperture (*o.*) and gives rise to a secondary oscular tube. When this has taken place, the reversed arrangement of the triradiates is that which is proper to an oscular tube, and in the normal relation to the water-currents. Woodland (1905, p. 268) states that as soon as an osculum is formed "the young triradiates immediately assume the oscular arrangement," a process of rearrangement which he explains on his theory that the triradiates are "righted" by incident stresses and strains. He has entirely overlooked the fact that the triradiates can take on the oscular arrangements before ever an osculum exists; hence his whole argument falls to the ground. Whatever explanation may be given as to the causes which have operated in phylogeny in producing definite spicular arrangements, it is clear from the facts adduced that, in ontogeny, the spicules may take on a special arrangement before the conditions exist, to which their arrangement is adapted.

I may say at this point a few words about Woodland's theory of the "righting" of the triradiate systems. He states that even in the oscular tube the young triradiates of Sycons have an irregular orientation, which is later corrected by the action of direct stresses and strains on the wall of the sponge (1905, p. 265, footnote; 1907 [2], p. 76). His observations do not agree with mine on various species of Leucosolenia. Both in the oscular tubes and diverticula, I have always found the youngest triradiates to have from the first the orientation normal to the region in which they are found. The best way to see this is to take pieces of sponges preserved in alcohol, mount them stained or unstained in Canada balsam, and examine them by polarized light with crossed prisms; all the spicules appear brilliantly illuminated and the young stages are clearly seen. I must express my entire scepticism with regard to Woodland's theory of "righting." I believe the youngest spicules take from the first the position they have when full grown. That, at least, is my impression from the oscular tubes of Ascons. I suggest that the irregular orientation of the young triradiates in oscular tubes of Sycons is related to the numerous, closely set diverticula (radial tubes), continually being formed, in which a special arrangement of the spicules prevails, just as in Leucosolenia; and that it is comparable to the regions (x) where in Leucosolenia also the orientation of the spicules is chaotic, in relation to beginning, or even to future, evagination of the body-wall. These chaotic regions might be expected to be far more numerous in a growing Sycon than in a Leucosolenia. Compare the long, smooth oscular tubes of Leucosolenia (Pl. 21, figs. 104—107) with the young Sycon depicted by Maas (1900 [2], pl. x, fig. 7); his figure recalls the classical Diana of Ephesus.

Woodland gives a figure (1905, p. 271, text-fig. 10) to illustrate his theory that the body-wall is pressed in between the arms of the triradiates, so that "a groove is formed in line with each ray of the spicule along which the apical cell must tend to travel." I can confirm the first part of these state-

ments to some extent; the full-grown triradiates, placed more towards the dermal surface, can be seen plainly to bulge out the dermal surface so as to produce depressed areas in the angles between the rays, in all Ascons (compare fig. 71 *b*). Unfortunately for Woodland's theory, however, this only applies to the full-grown spicules; the young triradiates develop far in, close to the gastral surface, where the superficial depressions do not reach, and where consequently no grooves can direct the apical cells in their untutored wanderings.[1]

IX. General Remarks on the Formation of Calcareous Spicules.

A spicule may be defined briefly as a sclerite, or skeletal element, of intra-cellular origin. The chief interest which

[1] I must point out that Woodland has occasionally fallen into some errors of statement in relation to objects which he has not himself studied. In one place (1905, pp. 238, 239, foot-note) he speaks of tuning-fork spicules in the stalk of Clathrina lacunosa. It is hardly necessary for me to point out that no such spicules occur in this sponge; Woodland was apparently thinking of the elbowed monaxons of the stalk, spicules of a very different type. Tuning-fork spicules are characteristic of the extinct Pharetronidæ and of some recent heterocœlous genera, such as Lelapia, probably allied to the Pharetrones; they are not known in any Ascon; and their position in the body of those sponges which possess them does not at all confirm Woodland's explanation of their form and characters. In another place (l. c., p. 274) Woodland states that "in no calcareous sponges do there coexist small monaxons . . . . with triradiates," but that either small and large monaxons, or large alone, are found. Now, the common Leucosolenia botryoides of our coasts, the earliest described species of Ascon, always has triradiates in great abundance, some of them very thick and massive; but its monaxons are frequently so small that they have been completely overlooked by spongiologists of repute, and require high powers to find them (compare Minchin, 1905 [**2**], text-fig. 97, p. 388). [Since this was written Woodland has corrected his statements concerning Clathrina lacunosa (1908, p. 148, footnote).]

Lapses of this kind are like the proverbial flies in the ointment; they weaken the theoretical conclusions which they are adduced to support, and they are a joy to the hostile critic. Woodland has himself produced such an abundant crop of valuable data upon spicule-formation that it is to be regretted that he did not rely more upon his own material as a basis for his speculations.

attaches to the study of spicule-formation arises from the fact that in some cases, at least, we have before us, as it were, the meeting ground of inorganic and organic forces. In calcareous sponges the spicules are almost entirely composed of calcite, which is a definite crystalline substance, and hence subject to laws which result from its physical structure. On the other hand, the spicules arise in living cells, and the forms which they assume are undoubtedly correlated with the bionomics of the organism rather than with the physical properties of the skeletal material. The fitness of the triradiate spicules for supporting the body-wall is obvious to anyone acquainted with the structure and life conditions of the sponge. On the other hand, the monaxons may be reasonably supposed to form an efficient protection to the sponge as well as, probably, contributing something to its support; while the gastral rays, curving up towards the oscular opening, very probably tend to keep out parasites, as Dendy has suggested, and I am quite unable to agree with Woodland (1905, p. 239) that they are entirely functionless.

Maas (1900 [1]) and Weinschenk (1905)[1] regard the spicule as the result of two activities, one organic-cellular, determining the form as a whole, the other inorganic-crystalline, responsible for the molecular structure of the contents. That this is, in a general way, a correct statement of the facts of the case no one can doubt. The chief question is the exact limitation between the respective spheres of influence of the vital and physical forces, and, in particular, whether, and to what extent, the form of the spicule may be influenced by its physical properties, that is to say, its crystalline nature.

In discussing this question we may begin by distinguishing

[1] "In every instant of the formation of a calcareous spicule, the deposition of the organically secreted calcite on the parts already present takes place according to purely inorganic laws of crystallisation, but there must always be present an organic element, which in its turn hinders the external inorganic form-production, and subordinates the external form to the conditions of the organism."—Weinschenk (1905, p. 584).

between primary spicules, derived each from a single mother-cell, which may divide to form formative cells, and aggregate[1] spicules, derived originally from several mother-cells, and representing a fusion of two or more primary spicules ancestrally distinct from one another. Instances in the Leucosoleniidæ of primary spicules are the monaxons, and of aggregate spicules, the triradiates and quadriradiates.

If we consider the monaxon spicules first it does not appear to me possible to correlate their form in any way with their physical, that is to say, their crystalline properties. To begin with, they always have unlike poles, which is a point of difference from any crystalline body, but which can be correlated with their situation in the body-wall, one end being embedded in the tissues, the other projecting free into the water. The small monaxons of L. complicata are straight, but in most cases the shaft is curved in such a way as to cause the free apex to project more vertically outwards into the water. The frequent presence of a distinct barb at the free distal end is a character which may be regarded as increasing the efficiency of the spicule as a protective weapon, and I would invite those who find such an explanation too teleological for their taste to put forward an alternative theory, and to explain the presence of the barb on mechanico-physical grounds.

If crystalline properties fail to explain the forms of the monaxons, we may inquire what other causes can have produced the results that are before us. Woodland (1905), if I understand him rightly, considers that the presence of two nuclei as two centres of cell-activity, between which the spicule arises, explains the monaxon form. But we do not find that all monaxon spicules arise between two nuclei. The monaxon spicules of calcareous sponges certainly appear to do so invariably, but those of siliceous sponges, according to the

[1] I prefer this term, suggested by Woodland (1907, p. 56), to the term secondary spicules used by me formerly (1906). Woodland uses the term "secondary" spicules for those which are partly formed by the action of adventitious cells, such as the Lithistid desmas.

unanimous testimony of all observers, including Woodland (1908), are produced, and built up entirely, each by a single cell. Woodland has also described typical monaxon spicules of certain molluscs as arising each in one cell (1907 [1]). In calcareous spicules we have some important analogies presented by the formation of the gastral rays of the quadriradiates. In considering this point there are two possible views to take with regard to the gastral rays; first, that they are sclerites of independent origin, not homologous at all with the monaxon spicules, in which case they do not furnish any analogy that can be used in my argument; secondly, that they represent, as I believe, monaxon spicules formed primitively in the interior of the oscular rim, which have become fused to the underlying triradiates. It may be asked, on this view, why monaxons formed on the dermal side did not also become fused to the triradiates. It is not easy always to suggest explanations for events that have occurred, but it is often still more difficult to explain why things have not happened that might possibly have taken place, and it can only be supposed that it is a more suitable arrangement for the monaxons that project on the exterior of the body-wall to be easily detachable structures, which are abandoned by their formative cells when completed. Now, if the homology of the gastral rays with monaxons be accepted, it is important to note that the gastral rays sometimes have one nucleus on them (Clathrina cerebrum), sometimes two, sometimes, as in Clathrina contorta, even four. On the ground of this analogy, as well as on more general grounds, I am inclined to think that the primitive sclerites from which the monaxon spicules of Calcarea arose were not originally formed between two nuclei, but by a cell with a single nucleus; and that the presence of two nuclei is a feature which was developed in correlation with the elongated, needle-like form, rather than antecedent to it.[1]

[1] Woodland explains away the difficulty, on his theory, of the single cell on the gastral ray by assuming a co-operation between the actinoblast of the gastral ray and the basal formative cells of the triradiate system.

If, however, we waive these objections and assume that it is a universal rule for calcareous monaxon spicules to arise between two nuclei, we are still, it seems to me, very far from explaining the forms these spicules possess. The twin centres of secretive activity would only furnish us with a more or less elongated, bi-polar, intra-cellular structure, a type of spicule, in fact, such as is commonly found in siliceous sponges. We should still require some other factors to explain the projection outwards from the body wall, the frequent curvature, and above all the barb. Woodland's theory supplies, as it seems to me, nothing more than the simple rod-like spicule, which would be required on any theory of evolution as the starting-point for further bionomic adaptation; the raw material, as it were, for the action of selection or of the natural method, whatever it may be, by which the structural characters of organisms are brought into harmony with their needs as determined by the conditions under which they live.

To sum up the conclusions which I have endeavoured to establish in the foregoing paragraphs, it seems to me that the forms of the monaxon spicules are in no way explicable by the physical properties of the material, and only to a very limited extent, if at all, as the direct mechanical outcome of the conditions under which they develop. The monaxon spicules owe the peculiarities of their form chiefly and, perhaps, entirely to their relations to the sponge-body, and are adapted to the needs of the organism.

We may consider now the aggregate spicules; these, in calcareous sponges, always take the form of triradiate systems, to which a fourth ray may or may not be added.[1] Here we have two main types to consider, the perfectly equiangular triradiate systems found in Clathrinidæ, and the alate or sagittal spicules, with one unpaired and two paired angles found in Leucosoleniidæ. It is sufficient

[1] As pointed out above, the large monaxon spicules of Clathrinidæ are probably to be regarded, in some cases at least, as aggregate spicules, perhaps derived from modification of triradiate systems.

for the present to confine the discussion to the spicules of Ascons.

In my former memoir (1898) I rejected the notion that physical factors, such as crystallisation, had anything to do with the forms of the triradiates, and considered their symmetry to be entirely the result of adaptation. I have been forced to reconsider my position in this matter. The regular and unfailing symmetry of the triradiates in the species of Clathrina is a very striking thing, and becomes more so when we take other facts into consideration. Von Ebner has shown that in Clathrinid triradiates the facial plane, i. e. the plane containing the apices of the three rays, is at right angles to the crystalline optic axis, so that the morphological axes of the three rays lie entirely in three planes of crystalline symmetry which contain the optic axis, and intersect at angles of 120°; and, further, that the straight gastral rays grow up in the optic axis, with which their morphological axis coincides. As Bidder (1898) has pointed out, the minute spines on the gastral rays of Clathrina cerebrum also form angles of 120° with one another, and repeat the symmetry of the basal rays. In the abnormal spicule figured by me (fig. 101) the additional rays come off from the main shaft at the same unfailing angle. If this angle is the result of adaptation, as I formerly argued,[1] it must be supposed that, by the operation of this principle over a vast time, the cells have acquired a hereditary tendency always to attach one spicule-ray to another at a fixed, invariable angle. This becomes difficult to imagine, when we find, as is frequently the case, that the three rays of a triradiate may be very irregular in the earliest stages, when they are still more or less separate from one another, and that they only become completely symmetrical when fused together; an observation directly opposed to the theory of hereditary tendency, which, as it seems to me, cannot go further than

[1] Maas, from his embryological observations, has pointed out that the form of the triradiates is in no way correlated with the arrangement of the pores, at least in ontogeny (1900 [2], p. 227).

to bring about simply the apposition of three mother-cells, and hence to produce three rays. It seems impossible to explain either by heredity or adaptation the definite relations of the rays to crystalline symmetry; some other cause must be sought for the equiangular arrangement. Woodland has explained the equiangular condition as due to development of the spicule "under undisturbed conditions" (1905, p. 270), while the sagittal triradiates are supposed to be the result of development in disturbed conditions.[1] I do not think this explanation can be accepted. Why should the spicules of a specimen of Clathrina coriacea, which lives between tide-marks and is subject to the rush of the tide four times in every twenty-five hours, roughly speaking, be supposed to develop under undisturbed conditions? Woodland has, moreover, confused together triradiates which are sagittal by deviations in the angles, and spicules which are sagittal simply by elongation of one ray, while remaining perfectly equiangular, as in Clathrina blanca and C. lacunosa. I find myself unable to attribute the wonderfully regular symmetry of the angles of the triradiates of Clathrinidæ either to adaptation, as I did formerly, or to the conditions under which the spicule develops, as Woodland does. I can only refer the regularity of the angles between the rays to the operation of the physical laws resulting from the crystalline properties of the material, which so modifies the growth of the rays relatively one to another, that it causes the three constituent elements of the compound spicular system to meet at the definite angles which are so constant a feature of these structures.

Various objections naturally occur to this view, which I will discuss. The first is, how is the symmetry of the Leucosolenia-triradiate to be explained, where equal angles between the three rays are the greatest exception, if

[1] I do not feel confident that I have quite grasped Woodland's meaning; the general trend of his argument seems to be that in the reticulate Clathrinidæ disturbances are balanced and do not preponderate in any one direction, as they do in the more erect Leucosolenii dæ.

indeed they ever occur.[1] Here we must consider the remarkable facts discovered by von Ebner concerning the relations of the triradiate systems to crystalline symmetry.

Von Ebner divides the triradiate systems of calcareous sponges into three main classes, which he terms perregular, sagittal, and irregular respectively. In the perregular triradiates, exemplified by those of Clathrinidæ, the optic axis is vertical to the facial plane of the rays, and all three rays are optically and morphologically equivalent[2] and meet at equal angles. In the sagittal spicules, seen in Leucosoleniidæ and Heterocœla, the optic axis is never vertical to the facial plane, and there are always an unpaired and two paired rays; the morphological axis of the unpaired ray lies in a plane of crystalline symmetry which contains the optic axis and halves the angle between the two paired rays. An irregular triradiate is defined as one in which no ray can be found, the morphological axis of which defines a plane of crystalline symmetry containing the optic axis and halving the angle between the other two rays; but since no irregular spicules, in this sense, could be discovered, it is doubtful if they exist at all, and it is not necessary to consider them further. The sagittal triradiates were further divided by von Ebner into primary and secondary forms. In the primary sagittal triradiates the morphological axes of all three rays lie entirely in planes of crystalline symmetry

[1] As von Ebner has pointed out, the three rays of a triradiate system seldom lie in one plane, but form the edges of a shallow pyramid, of which the base lies towards the gastral, the apex towards the dermal, surface. Hence a careful distinction must be drawn between the actual angles at which the rays join, and the apparent angles which they present in the facial projection, that is to say, when a spicule is seen under the microscope with its dermal surface uppermost and the apices of its rays resting on the slide. In the case of certain triradiates of Leuconia solida, von Ebner has shown that a spicule which appears regularly equiangular when viewed in the facial projection, may be, in reality, markedly sagittal if its real angles be measured accurately.

[2] One ray, as in Clathrina blanca and C. lacunosa, may be longer than the other two, without disturbance of other conditions. Von Ebner does not appear to have examined triradiates of this type.

which contain the optic axis and intersect at equal angles; so that the triradiates of this class, if projected in a transverse plane (that is to say, in a plane at right angles to the optic axis), appear as regular equiangular triradiates, whatever their actual form may be.[1] In the secondary triradiates, on the other hand, the projection in a transverse plane gives a figure in which the angle between the paired rays is greater than 120°, usually 150°—180°, and the curvature of the paired rays is not confined to a plane of crystalline symmetry which contains the optic axis. The secondary sagittal triradiates may be derived from the primary type by supposing that the paired rays rotate symmetrically away from each other, until they may come finally to lie in the same straight line when projected in a transverse plane of crystalline symmetry. An extreme form of the secondary sagittal type is seen in the "pseudo-regular" triradiates of the gastral surface of Sycortis quadrangulata; spicules which appear morphologically to be regular equiangular triradiates, but which are entirely different crystallographically from the true regular forms, since one ray, the unpaired ray, has its morphological axis coincident with the optic axis, while its paired rays both lie in one and the same plane of crystalline symmetry passing through the optic axis.

From the discoveries of von Ebner it is seen that the primary sagittal triradiates of Leucosoleniidæ and Heterocœla agree with the perregular triradiates of Clathrinidæ in so far, that both types alike appear equiangular when projected in a plane lying at right angles to the crystalline optic axis. With regard to the secondary sagittal forms, it can also be stated that their morphological symmetry is in a definite and constant relation to their crystalline structure. Ebner's investigations establish, so far as they go, the following

[1] This remarkable fact was also discovered by Bidder (1898), who regarded it as a universal law for all triradiate systems; Bidder was not aware, apparently, of the existence of von Ebner's secondary sagittal forms. In my paper at the British Association at York (1906) I also overlooked von Ebner's statements, and attributed the discovery to Bidder.

generalisation :—In all triradiate systems of calcareous sponges, whatever their form, there is one ray, the morphological axis of which lies entirely in a principal plane of crystalline symmetry, that is to say, a plane which includes the optic axis, and which also bisects the angle between the other two rays. Moreover, the ray which defines this plane of symmetry is the posterior ray, that is to say, the ray which, in the primitive Olynthus, points in the opposite direction to the oscular aperture, and therefore occupies, primitively at least, a definite position in the sponge-body. Thus, in the primitive orientation of the triradiate systems, such as is found to persist in the oscular tubes of Ascons, probably also in the oscular rim of any calcareous sponge, derived directly from the arrangement presented invariably by the embryonic Olynthus-form, the plane of crystalline symmetry defined by the posterior ray would also halve more or less accurately the entire sponge-body, since it would pass through the morphological axis of the body. The triradiate systems of the Calcarea exhibit a plan of crystalline symmetry which is in relation, not only with the morphological symmetry of the spicules themselves, but also with that of the sponge-organism. I think it may be reasonably inferred that this striking fact must be explained by physical peculiarities of the material, since it is a character of the spicules which cannot possibly have any biological or functional significance.

Maas (1904 [**2**], etc.) has advanced, as a proof that the form of the spicules is determined entirely by the organism in which they develop, the observation that in calcareous sponges grown in water deprived of $CaCO_3$, the sclerites, though not containing calcite, still show the triradiate form. I do not gather, however, from Maas's memoirs, that these organic triradiate spicules have a regular symmetry, but simply that they consist of three rays joined together. As I have pointed out above, the operation of a hereditary tendency is an adequate explanation up to this point, and, in my opinion, no further.

To sum up briefly my conclusions with reference to the spicules of calcareous sponges; it is my opinion that the forms of primary spicules are determined solely by their relation to the organism and in no way by their crystalline structure, but that when primary spicules are joined together to form secondary systems, crystallisation may be a condition determining the angles at which they join. So long as the optic axis is vertical to the facial plane of the rays, the angles between the axes of the rays can only be 120° in the facial projection; variations in the angles first become possible by the rays becoming, as it were, displaced from their primitive relations to the planes of crystalline symmetry.

The conclusions reached in the foregoing paragraph may perhaps, as I have argued elsewhere (1905 [1]), be applied also to the second of the three principal stems of the sponge-phylum, the Hexactinellida or Triaxonia; but in this case we must be more cautious, since there is no proof that in this group the spicules are crystalline in nature. The most striking feature of Hexactinellid organisation is the constancy with which the rays meet at right angles; and even when this peculiarity is masked by curvature or reduction of the rays, it is still shown clearly by the axial thread, as in the beautiful examples of the monaxons showing the "axial cross." Schulze (1887, pp. 501—504) first tried to give an explanation for the symmetry of the Hexactinellid spicule by ascribing it to an adaptation to the structure of the soft parts of the sponge, the triaxon spicule being shown to fit in perfectly between the thimble-shaped chambers suspended in the thick wall. As I have pointed out, it is highly probable that spicules were formed before chambers in the evolution of Hexactinellid sponges, in which case the form of the spicules could not have been determined by the arrangement of the chambers; and even if we assume that chambers were present before spicules, we do not get an adequate explanation of the triaxon form, since a layer of chambers, if disposed in the manner most economical of space, would tend to take on a honeycomb-like arrangement,

leaving interspaces to which an equiangular triradiate, not a cruciform spicule, would be the natural adaptation. Hence I consider that the constant rectangular junctions of the rays of Hexactinellid sponges cannot be regarded as an adaptation, but as an inherent peculiarity of the spicule itself, probably determined by the physical, if not the crystalline, properties of the material.

Strongly contrasting with the Hexactinellid spicules are those in the third sponge-stem, the heterogeneous assemblage united under the comprehensive name Demospongiæ by Sollas. Here the primitive spicule is apparently a tetraxon, or possibly an aster, of which the tetraxon is but one modification.[1] From this arises every possible type of form. Dendy has recently [2] constructed a phylogeny of the spicules of Demospongiæ. The only point which it is necessary for me to dwell upon here is, that in the Demospongiæ the angles at which the spicule-rays meet are infinitely variable, and show not the slightest tendency to be constant in any way. It is sufficient to mention the modifications of the triæne, distinguished by a formidable nomenclature (protriænes, orthotriænes, anatriænes, etc.). It is evident that whatever may be the case in Calcarea and Hexactinellida, the physical nature of the spicule material in Demospongiæ offers no obstacle to the indefinite variability of the spicules. I see nothing against accepting Schulze's theory (1887, p. 503) that the primitive tetraxon of Demospongiæ arose as an adaptation to the form of the interspaces between numerous closely-packed spherical chambers. Comparison of existing forms, such as Plakina and Oscarella, make it highly probable that in this stem forms with chambers preceded forms with spicules in evolution.

It may be a stumbling-block to many that in Calcarea and Hexactinellida physical conditions should be regarded as a

[1] In this connection the statement of Maas (1900 [3]), that the asters of Tethya arise by fusion of separate tetraxons, must not be overlooked; but compare Woodland (1908).

[2] 'British Association Reports,' York, 1906.

factor in controlling the production of forms of spicules, while in Demospongiæ adaptive influences are allowed exclusive sway. It is a natural tendency of the mind to seek for uniform explanations in parallel cases. But if we accept Schulze's three main stems, or rather branches, of sponge-ancestry, it is probable, indeed, almost certain, that in each stem the spicular skeleton was acquired independently, and might therefore have been subject to different controlling influences in each case.

With regard to the evolution of the spicules within the group of the calcareous sponges I have but little to add to the conclusions I put forth in 1898 (p. 568). I then regarded the triradiate system as a fusion of three primitively separate monaxon sclerites, and I am more than ever convinced of this, now that I have found that the primary monaxons develop in a manner perfectly similar in every way to a single ray of a triradiate. I regarded, and still regard, the ancestral form of spicule in Calcarea as "a simple monaxon placed tangentially, and completely embedded, in the body-wall," and I believe that the typical monaxon of Calcarea, as now occurring, for instance, in Leucosolenia, arose from the primitive type by a process of accretion at one end, causing the older portion of the spicule to protrude from the surface of the body as the distal projecting extremity. The triradiates, on the other hand, arose by fusion of three primitive monaxons; and as I have pointed out above, the fusion to form triradiates took place by that extremity of the sclerite which, in existing monaxons, projects from the surface of the body.

From von Ebner's results, with regard to the regular triradiates, I think we may add a further conclusion, namely, that the primitive, tangential monaxon had its crystalline optic axis at right angles to its morphological longitudinal axis; in fact, that the optic axis of the ancestral monaxon sclerite was vertical to the body-wall, that is to say, radial to the longitudinal axis of the primitive Olynthus. The projecting monaxons of existing Calcarea have, as von Ebner has shown, the morphological axis inclined to the optic axis,

the inclination being greater or less in different parts of the spicule, according to the curvature, which is always in a plane containing the optic axis; and it is a striking fact that at the distal extremity of the spicule the optic axis is at right angles to the morphological axis. In other words, the oldest part of the monaxon, the part formed when the spicule lies entirely in the body-wall, has its optic axis orientated in the manner which was, on my view, the primitive and ancestral one for the spicules of calcareous sponges.

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EXPLANATION OF PLATES 17—21,

Illustrating Mr. E. A. Minchin's paper on "Materials for a Monograph of the Ascons."

On Plates 17—20 all the figures, except Fig. 101 on Plate 20, are drawn to a magnification of 1000 linear.

PLATE 17.

Figs. 1—8.—Development of the small straight monaxons of Leucosolenia complicata, as seen in surface views of the wall of the sponge.

Fig. 1.—Scleroblast with two nuclei and commencing separation of the two formative cells. No trace as yet of spicule-formation.

Fig. 2.—Similar stage, but with the first foundation of the spicule indicated as a delicate line, passing obliquely between the two nuclei.

Fig. 3.—The delicate line of the last stage has expanded into a clear, fusiform area, sharply limited from the surrounding cytoplasm. No trace as yet of mineral deposit.

Fig. 4.—Similar stage to the last; the clear space is larger, and running through part of it a delicate axial line could be made out.

Fig. 5.—Commencement of mineral spicular deposit within the clear space, which it far from fills.

Fig. 6.—A later stage; the "thickener" beginning to travel away from the projecting distal extremity of the spicule.

Fig. 7.—Spicule nearly fully formed; the thickener quite close to the founder.

Fig. 8.—Spicule complete. The founder apparently wandering off, the thickener at the proximal extremity.

Figs. 9—18.—Development of the large curved monaxons of L. complicata, as seen in surface views of the wall of the sponge.

Fig. 9.—Lance-head between the two nuclei of the formative cells, the separation between which is as yet scarcely indicated.

Fig. 10.—Lance-head with the two formative cells at each extremity.

Fig. 11.—Similar stage; the lance-head much thicker; the shaft of the spicule growing rapidly in length.

Fig. 12.—Later stage; the thickener commencing to leave the completed lance-head.

Fig. 13.—The lance-head abandoned; the activity of the thickener shown by a distinct rim (*r.*) on the shaft.

Fig. 14.—Similar stage; the rim (*r.*) formed by the thickener very distinct. The founder in its progress has encountered a ray of a triradiate system (*r.t.s.*), producing a distinct kink in the spicule (*k.*).

Fig. 15.—Similar stage.

Fig. 16.—Similar stage in the variety of the large monaxons which is without a lance-head.

Figs. 17, 18.—Spicules nearly fully formed, showing the two formative cells close together at the proximal extremity of the spicule.

Figs. 19—29.—Development of the triradiates and quadriradiates of L. complicata as seen in surface views. The nuclei seen at a higher focus in the preparation are coloured more deeply than those seen at a lower focus.

Figs. 19, 20.—Two sextetts in which the first trace of spicule-formation is seen in the form of irregular granules. In one instance the granules are in a distinct vacuole.

Fig. 21.—Sextett containing three small pieces of calcareous matter, one larger than the others, and evidently taking shape as the unpaired ray.

Fig. 22.—Septett containing a large and distinct unpaired ray and traces of one of the lateral rays and of the gastral ray.

Fig. 23.—Sextett with small triradiate, lying in a clear space limited by a distinct sheath.

Fig. 24.—Small quadriradiate with six basal formative cells and gastral actinoblast with two nuclei.

Figs. 25, 26.—Small quadriradiates with still a single nucleus in the gastral actinoblast.

Fig. 27.—Small triradiate with six formative cells.

Fig. 28.—Older quadriradiate. The gastral actinoblast, with two nuclei, has a polygonal outline with incurved sides and sharp corners, caused by fitting in between the bases of the collar-cells.

Fig. 29.—A nearly full-grown quadriradiate with all six formative cells. Gastral actinoblast not drawn.

Figs. 30—36.—Derelict triradiates and quadriradiates, some with a few cells still attached, others with none. All occurring close to the dermal surface.

Figs. 37—41.—Histology and spicule-formation of Leucosolenia complicata, as seen in sections.

Fig. 37.—An ordinary collar-cell (on the left) and two sister-cells resulting from recent division. In the latter the collars are not yet formed, and the flagella are short, thickened at their base, and tapering to a point.

Fig. 38.—A cell of the dermal epithelium, mushroom-like type; the body of the cell, containing the nucleus, is placed far down the surface, and sends out delicate processes.

Fig. 39.—A cell of the dermal epithelium in which the nucleus has recently divided.

Fig. 40.—Two sister-cells of the dermal epithelium resulting from recent division.

Fig. 41.—Cells of the dermal epithelium passing in to form a spicule (compare Figs. 1 and 2).

PLATE 18.

Histology and spicule-formation of Leucosolenia complicata as seen in sections (collar-cells drawn in outline, except in Fig. 53).

Fig. 42.—Section showing dermal epithelium and a young monaxon spicule (*m.sp.*) with its two scleroblasts. No nuclei of epithelial cells come into the section. The other parts of the section are not figured.

Fig. 43.—Section showing a young monaxon spicule (*m.sp.*) with its two formative cells (the distal end of the spicule broken off); two cells of the dermal epithelium (*d.ep.*); five collar-cells, and amongst them an excretory cell (*ex.c.*).

Fig. 44.—Section showing a monaxon spicule (*m.sp.*) at a late stage of growth (compare Fig. 7); the spicule is much broken in cutting. Two cells of the dermal epithelium and three collar-cells are also seen.

Fig. 45.—Portion of a section through the region where the sponge is attached to an alga (*A.*); the dermal epithelium (*d.ep.*) becomes at this point very columnar; the mesoglœa is greatly thickened. *scl.t.* Scleroblast attached

to a fragment of a triradiate. *scl.m.* Two formative cells of a monaxon spicule, which has begun to be formed as a clear space (compare Figs. 2, 3, and 4 on Pl. 17). Four collar-cells are seen.

Fig. 46.—Section of the oscular rim, showing five cells of the dermal epithelium; in one of them a portion of the shaft of a monaxon spicule (*m.sp.*) is imbedded. The two uppermost collar-cells (*c.c.*) come into the section.

Fig. 47.—Portion of a section of the oscular rim, showing four cells of the dermal epithelium; in one of them is embedded a monaxon spicule, which is cut across.

Fig. 48.—Section of an early stage of one of the large, curved, barbed monaxons (compare Fig. 9 on Pl. 17). The section was thick; hence the spicule, which was placed obliquely in the section, is much foreshortened in the drawing.

Fig. 49.—Portion of a section showing two cells of the dermal epithelium (*d.ep.*); a wandering cell (*am.c.*); two cells of a sextett (*scl.*); and six collar-cells, in one of which only the base is seen in the section; besides fragments of spicules.

Fig. 50.—Portion of a thick section, showing a young quadriradiate; on the basal system five of the six formative cells are seen; the gastral ray (*g.r.*) projects between the collar-cells and bears an actinoblast with two nuclei (compare Figs. 24 and 28 on Pl. 17).

Fig. 51.—Section showing a very young quadriradiate; five of the formative cells of the basal system are seen, and the large, granular, gastral actinoblast (*g.act.*) with its single nucleus (compare Fig. 26 on Pl. 17).

Fig. 52.—Young quadriradiate, intermediate in size between those drawn in the two preceding figures. Four formative cells of the basal system are seen, and the gastral actinoblast has two nuclei.

Fig. 53.—Three collar-cells, drawn from a section, with a granular excretory cell (*ex.c.*) amongst them.

Fig. 54.—Section passing through a pore (*P.*). On the right of it are seen two dermal epithelial cells (*d.ep.*), a sextett (*sext.*), and a cell (*p.c.*) which may be either the principal portion of the porocyte, or a gastral actinoblast.

Fig. 55.—Section showing a young triradiate embedded in its sextett of formative cells (*sext.*).

Fig. 56.—Section through the lower part of the oscular rim. On the right an excretory cell (*ex.c.*) is seen amongst the collar-cells; one collar-cell (*c.c.*), of which only the base appears in the section, has been filled in, to show the difference between its cytoplasm and that of the excretory cell. On the left is seen the gastral ray (*g.r.*) of a quadriradiate; near it is a dermal epithelial cell, which sends out a prolongation ensheathing the gastral ray.

Fig. 57.—Section of the oscular rim; on the extreme right the first collar-cell is drawn in outline; on the inner (lower) side four cells of the dermal epithelium come into the section, but on the outer side none are cut through, since the section passes between them. *m.sp.* Monaxon spicule with two formative cells. *scl.* Scleroblast on the ray of a triradiate cut across. *ex.c.* Excretory cell.

Fig. 58.—Young triradiate from a section showing the unpaired ray in its full length, bearing the basal and apical formative cells (*b.f.c.*, *a.f.c.*). The two paired rays are cut off close to their origins, but show their basal formative cells.

Fig. 59.—Gastral ray of a quadriradiate, from a section showing the granular actinoblast with two nuclei.

PLATE 19.

Figs. 60—70.—Gastral rays of quadriradiates, from vertical sections of the body-wall of Leucosolenia complicata.

Fig. 60.—Young quadriradiate showing the unpaired basal ray, broken, with the two formative cells (*b.f.c.*, *a.f.c.*), and the gastral actinoblast (*g.act.*), with two nuclei.

Fig. 61.—Young quadriradiate showing two scleroblasts of the triradiate system and the gastral actinoblast (*g.act.*) with two nuclei, which have apparently originated by very recent division of the original nucleus.

Fig. 62.—Quadriradiate with the gastral ray (broken in two places) bearing the actinoblast with two nuclei, and close to the base, among the collar-cells, an excretory cell (*ex.c.*).

Fig. 63.—A quadriradiate, showing two formative cells of the basal system, and two nuclei on the gastral ray, near the base of which is seen an excretory cell (*ex.c.*), apparently connected with the gastral actinoblast by a protoplasmic process.

Fig. 64.—A gastral ray of a quadriradiate, showing the tip enveloped by the actinoblast, and with an excretory cell (*ex.c.*) attached, from which granules are being ejected. It was difficult to be quite certain if the actinoblast contained one or two nuclei; possibly one has been cut off.

Fig. 65.—Similar to the last; gastral actinoblast distinctly with two nuclei.

Figs. 66, 67.—Full-grown gastral rays, each enveloped in an actinoblast bearing two nuclei (*n.act.*, *n.act.*), and carrying two excretory cells (*ex.c.*).

Fig. 68.—Portion of a section through the oscular rim; the first collar-cell is seen on the extreme right. *g.r.* A gastral ray covered partly by the dermal epithelium (*d.ep.*), but not bearing an actinoblast or excretory cell.

Fig. 69.—A gastral ray, of which the actinoblast is apparently giving rise to an excretory cell.

Fig. 70.—A gastral ray, enveloped in an actinoblast with two nuclei, but without any excretory cells.

Fig. 71.—A portion of the body-wall, seen from the gastral aspect, with the collar-cells brushed away; the figure shows, besides spicules and their scleroblasts and cells of the dermal epithelium, two pores (*P.*), a sextett (*sext.*), three ordinary amœbocytes (*amc.*), and numerous minute amœbocytes (amc^1.).

Fig. 71*a*, 71*b*.—Portions of sections showing the proximal ends of large monaxon spicules (*M.SP.*) bearing each two formative cells (*f.c.*, *th.c.*). In 71*b* are seen also two cells of the dermal epithelium.

Fig. 72.—Surface view of the body-wall seen from the gastral aspect; only the dermal epithelium (*d.ep.*) and the porocytes (*p.c.*) are drawn, to show the way in which the epithelium lines shallow depressions, at the bottom of which the pores are found.

PLATE 20.

Figs. 73—100.—Development of the spicules of Leucosolenia variabilis, as seen in surface views of the body-wall.

Figs. 73—83.—Development of the small monaxons.

Figs. 73—75.—Formation of the two scleroblasts by division of a cell of the dermal epithelium.

Figs. 76—78.—Youngest stages of the spicules; the lance-head embedded in the "thickener;" the shaft, as yet scarcely formed, is being laid down by the "founder."

Figs. 79—81.—The thickener leaves the fully formed lance-head and is travelling down the shaft, building it up to its full thickness; the founder prolongs the spicule at its proximal end.

Figs. 82, 83.—Monaxons nearly full-grown, with the formative cells close together at the proximal end of the shaft.

Figs. 84—86.—Three stages in the formation of medium-sized monaxons. Fig. 84 is a young stage, showing the lance-head still embedded in the thickener. Figs. 85 and 86 show spicules nearly full-grown, each with the formative cells close together at the proximal end of the shaft.

Figs. 87—89.—Final stages in the formation of the large-sized monaxons. In 87 and 88 the two formative cells are still present on the proximal end of the shaft, which tapers evenly to a sharp point. Fig. 89 is a spicule completely formed; the proximal extremity tapers abruptly and bears no formative cells.

Figs. 90—100.—Development of the triradiate and quadriradiate systems.

Figs. 90, 91.—Sextetts. In Fig. 91 the small size of the nuclei indicates recent origin by division from the three actinoblasts.

Figs. 92—95.—Four young quadriradiates, drawn from the dermal side; the spicules are slightly corroded by the glycerine. On each ray are seen the two formative cells, basal "thickener," and apical "founder." The gastral actinoblasts, being under the spicules, are not clearly seen, but can be made out to contain each a single nucleus. In Fig. 94 two cells of the overlying dermal epithelium (*d.ep.*) are drawn.

Figs. 96—100.—Rays of triradiate and quadriradiate systems, showing the migration of the "thickener" to the apex of the ray and the disappearance of the "founder." Figs. 98 and 100 are full-grown spicules, having the thickeners on the extreme points of the rays.

Fig. 101.—Abnormal triradiate of Clathrina coriacea. × 1250 linear. One of the rays has branched into two, each branch bearing a scleroblast.

Figs. 102, 103.—Amœbæ found commonly on the exterior of Leucosolenia variabilis. × 1000 linear.

PLATE 21.

Spicules, etc., of Leucosolenia lieberkühnii from Naples. Figs. 104—107, natural size. Figs. 108—122, × 170 linear.

Figs. 104—107.—Specimens of the sponge. Fig. 107 shows an unusually large oscular tube.

Fig. 108.—Distal end of a diverticulum; on the surface only the triradiate systems and pores are drawn; at the edge, in optical section, only the monaxons are shown.

Figs. 109—112.—Triradiates, isolated.

Figs. 113, 114.—Quadriradiates.

Figs. 115—119.—Ordinary monaxons.

Figs. 120—122.—"Walking-stick" monaxons. In Fig. 122 the distal extremity is shown more highly magnified.

On Mœrisia lyonsi, a New Hydromedusan from Lake Qurun.

By

Charles L. Boulenger, B.A.,
King's College, Cambridge.

With Plates 22 and 23.

Introduction.

During the months of March, April, and May of last year Dr. Cunnington and I were engaged in making a biological survey of the Birket el Qurun, a large lake in the Fayûm province of Egypt.

It is situated on the edge of the Libyan desert only a few miles from the spot where the wonderful fossil mammals have recently been discovered.

Of late years much attention has been devoted to the geology and topography of this interesting district, but nothing was known of the invertebrate fauna of the lake; it was to furnish the desired biological information that we were invited by the Egyptian Survey Department to make an investigation in the spring of this year.

The Birket el Qurun has a very unique interest as the remains of the historic Lake Mœris which was used as an artificial regulator of the Nile floods by the monarchs of the twelfth Dynasty.

At the present day the lake, which is about the size and shape of the Lake of Geneva, communicates with the Nile by means of a network of canals which irrigate the Fayûm,

but except during high Nile receives very little water. There is no outlet, the waters of the lake are therefore decidedly brackish. A general summary of the results of our investigations was published in a letter to 'Nature' last August. With the exception of the Hydromedusan which forms the subject of this paper, of Cordylophora, and of a ctenostomatous Polyzoan, resembling Victorella, which occurs in great abundance, the fauna seems essentially a freshwater one, composed probably of such inhabitants of the Nile as can accommodate themselves to the salinity of the water.

The hydroid stage of Mœrisia was obtained for the first time at the beginning of April. As medusa-buds were present on the hydranths we carefully watched for free-swimming medusæ; these, however, did not appear in our tow-nettings until May, when they first began to swarm.

Both stages were exhibited by me at the meeting of the Zoological Society of London held on June 18th, 1907, when the name Mœrisia lyonsi, gen. and spec. nn., was proposed for this new form.

I desire to take this opportunity to express our sense of deep gratitude to Captain H. G. Lyons, F.R.S., and his assistants at the Egyptian Survey Department, who spared no trouble to ensure the success of our expedition; Mr. Dowson, Mr. A. Lucas, and Mr. Dickinson were of the greatest assistance to us during our stay in Cairo, helping in the choice of servants and stores, and in the transport of our baggage to the Fayûm.

Mr. Dickinson, in addition, accompanied the expedition down to the lake, where his thorough knowledge of the district was invaluable and saved us much time and trouble.

Although commenced at Cambridge the greater part of this work was carried out in the Zoological Laboratory of the Oxford University Museum, and I wish to express here my sincere thanks to Professor G. C. Bourne for all the facilities which he afforded me.

I have also to acknowledge my indebtedness to Mr. C.

Martin who very kindly assisted me in making out the structure of the different forms of nematocysts.

To Professor Sir Ray Lankester I wish to convey my thanks for the interest he has shown in this publication, and for allowing me to engage a skilled artist for the preparation of one of my plates.

I. The Hydroid.

A. Anatomy.

The hydroid stage of Mœrisia was obtained on several occasions by means of a light dredge from the surface of the mud which covers the lake-bottom. Here at depths varying from six to fifteen feet it forms curious tangled-looking colonies usually growing on branches of Cordylophora (fig. 1).

The hydrorhiza is inconspicuous and consists of a short stolon-like tube of cœnosarc invested by a delicate perisarc which is slightly annulated and has a very dirty appearance, due to the inclusion in its substance of particles of mud and other foreign matter (fig. 2, *Per.*). From it are given off, indiscriminately in all directions, long, filiform branches which bear hydranths at their distal extremities and represent the hydrocaulus of the colony.

The free end of the hydrorhiza usually narrows out to form a slender stem also bearing a hydranth.

The proximal part of the branches are covered by the annulated perisarc; this is continued as a smooth, thin membrane which loses itself a short distance up; the remainder is quite naked.

The curious appearance of the colonies is due to this want of rigidity in the hydranth-bearing stems; the latter may attain the comparatively great length of over 1 cm., and become entangled both with neighbouring branches and with the Cordylophora on which the animal grows.

The hydranths attain a length of over 2 mm.; they may

be described as claviform, but their shape varies according to the degree of elongation or contraction; older specimens, also, bearing medusa-buds often have a greater relative diameter than younger ones.

Distally the hydranth is provided with a prominent cylindrical hypostome which is not constricted at its base. At the summit is the large mouth-opening surrounded by a narrow lip. At the proximal end the body tapers off gradually and passes imperceptibly into the narrow stem.

Each hydranth bears a small number of long, filiform tentacles arranged in a circle around the broadest part of the body below the hypostome; they seem to develop singly. The number is very variable, and an individual may bear from 0 to 8 tentacles; the majority, however, possess 4 or 5.

When fully extended the tentacles are very slender, and may attain a length greater than twice that of the hydranths on which they are borne.

The ectoderm of the hydranth consists chiefly of largish epithelial cells, the basal portions of which are provided with conspicuous longitudinal muscle processes. Small interstitial cells are to be found between the larger cells, and nematocysts occur in abundance. The ectoderm is deepest in the tentacle-bearing region of the body; in the hypostome it becomes much thinner, but again thickens around the mouth, where it forms the circular lip, crowded with large nematocysts. The ectoderm of the tentacles is continuous with that of the body; the cells are, however, less regular in shape, and form ring-like thickenings on which the thread-cells are borne.

The cells of the endoderm are by no means so uniform in structure as those of the outer layer. Roughly, three regions may be distinguished, corresponding well with those described by Hardy (1) in his paper on the histology of Myriothela phrygia. These regions are:

(1) An oral region, situated in the hypostome; here the endoderm presents four (rarely five) conspicuous longitudinal ridges formed by the greater elongation of certain of the

constituent cells, which are of two kinds:—(*a*) Elongated conical cells with finely granular protoplasm and a small nucleus situated in the tapering basal part; (*b*) narrow palisade-like cells, between the distal ends of which the former are wedged in; the protoplasm is clear and scarcely stains; their bases bear transverse muscle processes embedded in the mesoglœa.

(2) The second region is that from which the tentacles arise. The palisade cells are replaced by strongly vacuolated cells likewise arranged in longitudinal ridges; the latter are, however, less conspicuous than in the oral region, and are, moreover, far more numerous; as many as twenty may be counted in a transverse section of a large hydranth. Between the vacuolated cells are situated numerous very characteristic club-shaped gland-cells with large nuclei and coarsely-granular protoplasm.

The endoderm of the tentacles is continuous with that of the gastric cavity; in a transverse section of these organs we see five or six large clear cells surrounding a small but distinct tentacle cavity (fig. 8, *Tent. Cav.*). In this respect Mœrisia resembles Hydra and differs from all known colonial hydroids, for in these the long axis of the tentacle is occupied by a solid core of large vacuolated cells of so-called "notochordal" structure.

(3) In the third region, at the narrow base of the hydranth, we find cells very similar to those described as occurring in the tentacle zone. The ridges have, however, become quite inconspicuous and gland-cells are very rare.

As before mentioned, nematocysts occur scattered about in the whole of the ectoderm of the hydranths. Four kinds can be distinguished, similar in most respects to the forms which have been described in Hydra (2). They are:

(1) A large, oval, barbed nematocyst (fig. 9, *a*, *b*) found in great abundance on the body, hypostome and tentacles; most numerous in the ectodermal lip which surrounds the mouth opening. When everted the thread is seen to possess a thickened basal portion on which are developed a proximal

whorl of three large barbs and two or three distal whorls of much smaller ones.

(2) A smaller barbed form, similar in all respects to that just described, but attaining only half the size. It is found in the same positions as the larger kind.

(3) A cylindrical nematocyst of slightly greater length but same diameter as (1), without barbs. The thread before eversion is coiled round the imaginary longitudinal axis, forming several coils (fig. 9, *c*). This form differs from the corresponding nematocyst of Hydra in the character of the thread, which is thicker; the coils, moreover, are further apart and fewer in number. This nematocyst is rare, and I have only come across a few examples in the tentacles.

(4) A small pip-shaped form, devoid of barbs, occurring in large numbers, chiefly in the tentacles. The thread is thick and short; it differs from that of the cylindrical nematocyst in being coiled round the transverse axis of the organ (fig. 9, *d*. *e*.).

B. Medusa-buds.

On the larger hydranths medusa-buds are to be found scattered about the broadest part of the body, between and below the bases of the tentacles (fig. 6, 7, *Med*.). They arise in the usual manner as hollow outgrowths of the wall of the gastric cavity. Their development seems quite typical; the future umbrella cavity makes its appearance in the ectoderm at the apex of the bud, and by its growth causes the approximation of the endodermal walls of the latter. This leads to the formation of the endoderm lamella and the radial canals. Manubrium, velum, and other organs arise in the usual way. During the later stages of its development the medusa is borne on a short slender stalk.

The four-rayed symmetry so characteristic of the adult can be made out early, and the rudiments of the tentacle-bulbs are large and conspicuous. The tentacles themselves do not seem to be formed until after the liberation of the medusa.

c. Reproduction by Lateral Budding.

In addition to the young medusæ many of the hydranths bear a small number of oval buds attached by short peduncles to the parent body (figs. 2, 6, 7, *Lat. B*[1].). They are usually developed from the proximal region of the hydranth, but occasionally some may be found in a more distal position, either between the tentacles or at the base of the hypostome.

The buds arise as hollow outgrowths of ectoderm and endoderm, which later become constricted at their bases. The endoderm at the place where such a bud is formed is considerably modified, the cells becoming almost amorphous and charged with an enormous number of yolk-like granules, which probably form a reserve of nutritive substances. The mesoglœa in this region is thin and inconspicuous; the ectoderm, however, is not much modified, and contains numerous nematocysts.

The buds occasionally develop one or two tentacles (fig. 6, *Lat. B.*); for this reason they may possibly represent much modified hydranths. Normally they become completely detached from the parent body. I have found several liberated buds of this type, some of which had secreted a gelatinous ectodermal investment; their fate I have been unable to ascertain.

Such a process of asexual reproduction is, I think, quite unique; the nearest approach to it seems to occur in Myriothela. In this genus, according to Hardy (1), oval buds are developed at the junction of stolon and body; occasionally an isolated one may be found higher up in the tentacle-bearing region.

Their development, as described by the above-named author, is also similar to that of the lateral buds of Mœrisia, and we find the same modification of the endoderm cells. In Myriothela, however, the buds develop directly into daughter-polyps before becoming detached, although connection with the cavity of the parent is lost at an early stage.

In Mœrisia we occasionally find a lateral bud developing in situ; it increases in size, and forms what must be a modified hydranth of very irregular shape, usually with one or two tentacles, but without mouth or hypostome (figs. 5 and 6, *Lat.* B^2.). From this are developed one or more hydrorhizal tubes with perisarcal investment, which produce lateral branches; at the extremities of these ordinary hydranths are borne (fig. 5, *Hydr.*). We thus get the curious appearance of a small colony growing from the body of a hydranth.

In some cases the lateral bud produces a hydranth without first forming a hydrorhiza (fig. 6).

d. Reproduction by Transverse Fission.

In Mœrisia we observe yet another kind of asexual reproduction, which may be regarded as a modification of the phenomenon of decapitation described in so many hydroids. Decapitation seems to be of frequent occurrence in the colonies of Mœrisia; the basal part of a hydranth becomes constricted off from the branch which bears it, and eventually falls off. Before this actually happens we can often notice that the branch has become considerably swollen below the constriction, and is on the way to becoming a new hydranth. This is probably repeated several times during a season; Dalyell (**3**), why first described the phenomenon, noticed that in Tubularia decapitation could occur as often as seven times in sixty-six days.

In several colonies of Mœrisia observed by me the whole process was being hurried over, and, even before the first hydranth was completely separated off, a series of constrictions had appeared on the stem below it, giving rise to a string of more or less spherical buds (fig. 7, *Strob. B.*). These, no doubt, become detached, and develop into new colonies.

Chun (**4**), discussing the phenomenon of decapitation, has suggested that it may represent a process of very unequal,

transverse fission, perhaps analogous with the monodisc strobilisation which occurs in some members of the Acelephæ (e. g. Cotylorhiza). Polydisc strobilisation, he thinks, may have developed as a later modification. To quote his words : " Was bei den Tubularien über einen längeren Zeitabschnitt sich vertheilte das finden wir bei der polydisken Strobilisation zeitlich zusammengezogen und in dem Auftreten mehrerer Ringfurchen am oralen Pole ausgeprägt."

Chun's suggestion seems, therefore, to be a very happy one, for in Mœrisia we find strobilisation no doubt derived from a process of decapitation, and what was probably a phenomenon initiated as a method of getting rid of old hydranths has developed into a most efficient method of asexual reproduction.

Murbach (5) has observed a somewhat similar form of transverse fission in Hypolytus, a curious solitary hydroid from Wood's Holl, Mass., U.S.A. In this form, however, the strobilisation occurs at the aboral, free end of the unattached stem; the liberated buds develop directly into new hydroids.

II. The Medusa.

A. Anatomy.

The liberated medusa (figs. 3, 4) has a globular umbrella, the height of which is about equal to the diameter; this varies in the different individuals, but never much exceeds 4 millimetres.

The umbrella is eminently contractile, and serves as a powerful organ of locomotion, the medusa being propelled through the water by alternate dilatations and contractions of its cavity.

Except for four bright red ocelli situated on the tentacle bulbs the medusa is quite colourless and transparent; when swimming about in the water it is almost invisible.

The umbrella is thin in a young individual, but as the animal

approaches maturity the jelly or mesoglœa increases in thickness, especially in the apical region where it may attain a depth equal to nearly one half the total height of the medusa. The ex-umbrellar surface is perfectly smooth and devoid of groups of thread-cells.

The velum is broad and muscular, and is a very conspicuous feature of the jelly-fish.

The digestive sac or manubrium varies in shape according to age; it is, however, always short, and even when fully extended never reaches much further than the middle of the umbrella cavity.

The mouth is approximately circular and is simple, being devoid of oral lappets or appendages such as occur in so many families of Anthomedusæ.

Sections of the distal portion of the manubrium show this organ to be approximately cylindrical in shape.

The endoderm is seen to form four very conspicuous ridges, inter-radially situated, extending along the whole length of the manubrium as far as the base of the stomach proper. In the oral region the individual cells are tall and narrow with abundant finely-granular protoplasm, and a basally situated nucleus.

These cells pass gradually into a second kind of endoderm-cell which lines the cavity of the central region of the manubrium; in these the nucleus occupies a central position, and divides the cell into a basal portion with clear protoplasm and a peripheral portion with finely granular contents which stain deeply. Wedged in between these cells are occasional club-shaped gland-cells with large nuclei and coarsely granular protoplasm.

In the stomach or proximal region of the digestive sac the inter-radial ridges are less conspicuous, and the endoderm lower; the cells are much vacuolated, and many are modified as gland-cells.

In a medusa which has not long been liberated this proximal region is seen to be considerably swollen.

The swelling becomes accentuated in slightly older speci-

mens, and examination shows this to be due to the formation of four deep longitudinal pouches (fig. 3) situated per-radially and giving this part of the manubrium the form of a cross when viewed from above or below.

From the top of the pouches four radial canals are given off which traverse the umbrella and terminate on the margin where their cavities become continuous with the cavity of the circular canal. These canals are lined by a very low epithelium consisting of very small, clear, cubical cells continuous with similar though slightly larger cells which cover the exumbrellar wall of the stomach.

The gonads are to be found on the walls of the stomach; the generative cells are distributed over the whole of the ectoderm of this region of the digestive sac, and attain their greatest thickness in the concavities between the per-radial pouches.

When we examine an adult medusa we find that the stomach has increased in size and has changed considerably in shape. The four per-radial pouches have become drawn out into long finger-shaped diverticula which extend more than half-way down the sub-umbrella (fig. 4).

Except at the distal extremities, the cavities of these diverticula are continuous with the cavities of the radial canals.

The gonads extend onto the walls of the diverticula and attain at this point a considerable thickness.

In the adult medusa we thus find an arrangement of the gastro-vascular system very similar to that described by Browne (**6**) in Willsia; as in that genus the study of immature forms proves the gonadial diverticula to be parts of the stomach. Sections (fig. 10) show that the outer or exumbrella walls of the diverticula are lined by small endoderm cells identical in all respects with those in the radial canals, whereas the remainder of the lining is formed by larger and taller cells directly continuous with and merging into the endoderm cells at the base of the stomach.

All the specimens examined by me were of the male sex;

it seems therefore probable that the female medusæ are liberated later in the year than the males.

The structure of the testis is quite typical, and in it the three tissue-zones described by the Hertwigs (7) are well developed. For a detailed description I must refer to the writings of these authors and to the paper by Günther (8) on Limnocodium, where a very clear account of the histology of the male organs is given.

As mentioned above, the tentacles, four in number in a normal individual, are given off from the umbrella edge at the terminations of the radial canals.

These tentacles are very slender and of great length when fully extended, the latter being equal to more than twice the umbrella.

At their bases the tentacles are swollen to form very conspicuous ocellar bulbs, each of which bears on its ex-umbrellar surface, a bright red ocellus. Each ocellus consists merely of a cup-shaped mass of pigmented sense-cells surrounding a number of clearer cells flush with the external surface of the bulb.

The tentacles are hollow, their cavities being continuous with that of the circular canal. The ectoderm forms wart-like thickenings arranged in transverse rings which become very conspicuous and almost bead-shaped when the tentacles are fully elongated (fig. 4).

All four kinds of nematocysts described as occurring in the hydroid were found also in different parts of the medusa. The barbed kinds are to be found scattered in the ectoderm of the whole of the manubrium, ocellar bulbs, and tentacles. In the manubrium they are especially numerous in the ectoderm around the mouth-opening, but they occur also in the more proximal parts, and are occasionally developed on the perradial gonadial pouches.

Sections of the manubrium revealed quite a large number of these nematocysts in the endoderm of the whole of that organ, situated always in the basal parts of the individual cells.

The two barbless kinds of nematocysts seem to occur chiefly on the ocellar bulbs and on the tentacles; as in the hydroid, the smaller pip-shaped kind was found in great abundance; the cylindrical form, on the other hand, was very rare.

The nematocysts of the hydroid and of the medusa were carefully examined and compared at the suggestion of Prof. Minchin; it seemed of great interest to find out to what extent these structures correspond in the two stages of a Hydromedusan. This is a subject which has been much neglected by students of the group, and I am not aware that any such comparison has previously been made. The fact that the nematocysts are identical in both stages of Mœrisia is somewhat important, and it would be interesting to know whether this is also the case in other genera. In the Hydromedusæ so little is known about the connection between the two stages and the assignment of medusæ to hydroids is often only a matter of inference; if it could be proved that the nematocysts are as a rule identical in the medusa and hydroid of the same species we would have a most excellent test to apply, and many interesting problems might be solved in this way.

B. Variation.

Dr. Cunnington and I were fortunately able to collect and preserve a large number of the medusæ, and on examination there proved to be much variation in the number of radial canals, gonads, and tentacles. I have counted these organs in 400 individuals, and of these 55, or nearly 14 per cent., differed from the type described above.

Of these abnormal specimens, eleven exhibited variation in the general symmetry of the medusa; one possessed only three radial canals, three gonads and three tentacles, nine had five, and one six of these structures.

This type of meristic variation is known to occur frequently among Medusæ, and I must refer the reader to the papers of Agassiz (**9**) and Browne (**10**) for further information on this subject.

The remaining forty-four abnormal individuals had the typical number of radial canals and gonads, but possessed additional tentacles in various positions on the umbrella edge. In most cases these additional tentacles were so arranged as not to destroy the radial symmetry of the medusa; ten individuals had four inter-radial as well as the four per-radial tentacles, whilst as many as twenty-eight possessed, in addition, eight adradial ones, thus making a total of sixteen tentacles.

The remaining six individuals were asymmetrical, the asymmetry being due to the development of an incomplete number of adradial and subradial tentacles.

I have given below in tabular form a detailed account of the number and arrangement of the radial canals in the 400 individuals examined.

Variation in the Medusa.

Table Showing the Number and Arrangement of the Radial Canals and Tentacles in 400 Individuals.

Number of Individuals.	Number of Radial Canals.	Number of Tentacles.				
		Per-radial.	Inter-radial.	Adradial.	Subradial.	Total.
1	3	3	—	—	—	3
345	4	4	—	—	—	4
1	4	4	1	—	—	5
1	4	4	1	2	—	7
1	4	4	—	1	—	5
2	4	4	2	4	—	10
10	4	4	4	—	—	8
28	4	4	4	8	—	16
1	4	4	4	8	6	22
9	5	5	—	—	—	5
1	6	6	—	—	—	6
Total 400						

It is a well-known fact that many medusæ which in the adult condition possess a large number of tentacles commence

life with only four per-radial ones; in such cases the number of tentacles would increase with the age, and therefore with the size of the individuals. This certainly is not the case with the medusæ which I have been describing, for in these they are chiefly the smaller individuals which possess supernumerary tentacles, the percentage of variability being very different in the smaller and larger specimens.

In order to show this point I divided the medusæ which I examined into two groups :—(*a*) With an umbrella-diameter varying from ½ to 2 mm., and (*b*) with a diameter measuring from 2½ to 4 mm.

Group (*a*) contained 278 individuals, of which 39, or about 14 per cent., had supernumerary tentacles, whereas of the 122 larger forms belonging to group (*b*) only 5 possessed such structures.

I think the figures show clearly that the multi-tentacular forms must be considered as exceptional varieties; from such small numbers it would not be fair to argue that some sort of natural selection is in progress.

III. Systematic Position.

The systematic position of Mœrisia lyonsi, unlike that of the other known lacustrine medusæ, presents no difficulty. The globular shape and four-rayed symmetry of the umbrella, the manubrial gonads, and the absence of otocysts refer the medusa to the Order Anthomedusæ.

The gymnoblastic hydroid stage confirms this position. The simple mouth, the four unbranched tentacles, and the narrow radial canals are a combination of characters which exclude Mœrisia from Haeckel's (**11**) families Tiaridæ, Margelidæ, and Cladonemidæ, and refer it to the only remaining family, that of the Codonidæ, to certain genera of which (e.g. Sarsia) it bears a striking resemblance.

The arrangement of the gonads in the adult is rather different to that of the typical members of the family. The study of young individuals, however, removes this difficulty,

for in them we find the generative cells regularly distributed over the whole of the base of the digestive sac.

It is a more difficult matter to find a position for the hydroid stage among the numerous families of the Gymnoblastea, certain of the characters, for instance the hollow tentacles and the methods of asexual reproduction, being quite unique among colonial Hydrozoa.

On the whole the family to which it bears the greatest resemblance is that of the Bougainvilliidæ, the points of similarity being the single circlet of filiform tentacles and the cylindrical hypostome not constricted off from the body of the hydranth.

The new genus may be defined as follows :—

"Hydrocaulus consisting of long unbranched stems rising at short intervals from a small horizontal hydrorhiza, the latter invested by a delicate annulated perisarc continued onto the bases of the stems.

"Hydranths claviform with a small number (commonly four or five) of hollow filiform tentacles arranged in a circlet around the thickest part of the body.

"Hypostome cylindrical, not constricted at its base. Asexual reproduction by budding and transverse fission.

"Medusa developed from the body of the hydranth; when liberated globular with four unbranched radial canals and tentacles. Mouth simple. Manubrium very short; the stomach region provided with per-radial pouches which in the adult are produced into finger-shaped diverticla extending down the sub-umbrella. Gonads developed on the whole surface of the stomach and its diverticula."

IV. Conclusions regarding Origin.

The occurrence of a new medusa in the waters of the Nile system is of great interest. It is true that the water of Lake Qurun is decidedly brackish, and that therefore the term freshwater medusa cannot with strict accuracy be applied to

Mœrisia, yet the salinity is known to have varied greatly even in historic times, and at the time when great cities such as Dimeh were constructed on the north side of the lake there is little doubt that the Birket el Qurun contained fresh water. Even at the present day, although the amount of salt is sufficient to make the water unpalatable, an analysis showed that at the west end of the lake (where the concentration is greatest owing to the distance of the feeder canals) the total salts amounted to only 1·34 per cent., of which ·92 per cent. was sodium chloride.[1]

At present, excluding Mœrisia, only three genera of medusæ have been described from the freshwater systems of the different continents. These are: Limnocodium, originally described from the tanks in the Regent's Park Botanical Gardens; Limnocnida, from Central Africa; and last the medusa of Microhydra recently found in North America.

An admirable summary of the structure of these forms has recently appeared in the 'Quarterly Journal of Microscopical Science,' vol. 50, to which I must refer for more detail (**13**).

Other medusæ have from time to time been recorded from lagoons in close proximity to the sea, but such forms, e.g. von Kennell's Halmomises (**14**) from Trinidad, and Annandale's Irene (**15**) from the Ganges Delta, cannot be regarded as belonging to the true freshwater fauna.

As in the case of the above-mentioned medusæ, the question arises: How did a marine organism like Mœrisia find its way into Lake Qurun?

Before attempting to solve this question a little more detail of the topography and geology of the Fayûm must be given.

Lake Qurun measures at the present day approximately twenty-five miles by six; it lies in a depression on the border of the Libyan desert, its waters being nearly 140 feet below the level of the sea.

The Fayûm depression is separated from the Nile Valley

[1] Quoted by Beadnell from a note by Schweinfurth in Willcock's 'Egyptian Irrigation.'

by a desert ridge varying in width from one and a half to six miles.

The Lake is connected with the Nile by means of the Bahr Yusuf; this is a canal about 200 miles long, which leaves the river near Assiut, and, passing through a break in the desert ridge, flows into the Fayûm where it divides into numerous branches, some of which discharge their superfluous water into the Birket el Qurun.

At the present day Lake Qurun is situated about 150 miles to the south of the Mediterranean Sea; the Nile is a fast flowing river, and it is highly improbable that a medusa could have made its way upstream as far as the Fayûm; it is far more likely that Mœrisia is a relic of the sea, which in past times is known to have covered that part of Egypt in which the Lake is situated.

The geology of Lower Egypt has fortunately been most carefully worked out in recent years, and on perusal of what has been written on the subject it is not difficult to imagine how this can have happened.[1]

In early Pliocene times the valley of the Nile and the Red Sea were not yet in existence, and the Mediterranean covered the whole of Egypt to a little further south than Cairo.

At a slightly later period violent dislocations took place in the position of the future Nile Valley, and a marine fjord was formed in that district reaching as far south as the 24° of latitude. Into this fjord opened several rivers coming from the east over what is now the Arabian Desert, and there is no doubt that its waters were brackish.

The Fayûm depression was formed separately towards the same period, i. e. late Pliocene, and was occupied by a large brackish lake connected with the Mediterranean on the one hand, and with the fjord of the Nile Valley on the other. It seems probable that at this time Mœrisia, as well as

[1] The following account of the geology of Egypt has been compiled chiefly from the works of Beadnell and Blanckenhorn; references to these are given in the bibliography at the end of this paper.

Cordylophora and the Polyzoon, first established themselves in this brackish lake.

The course of events in Pleistocene times is at present rather obscure; it is thought, however, that this large prehistoric lake became disconnected both from the sea and from the Nile fjord, and that the greater part of it gradually evaporated, leaving probably only a small lake on the site of the present Fayûm depression, the latter already separated from the Nile fjord by a rocky ridge.

In later Pleistocene times, probably in consequence of slight elevation, the fjord became gradually silted up, and the early Nile cut itself a channel through the lacustrine beds.

The Nile at this period Beadnell (**16**) supposes to have been flowing about 20 metres higher than at present; in some way or other its waters must have broken through the dividing ridge and the Fayûm depression again became converted into a large lake.

When this occurred there must have necessarily been a mingling of the fauna of the old brackish lake and that of the early Nile; most probably the greater part of the former was exterminated by the influx of fresh water, Mœrisia, Cordylophora, and the ctenostomatous Polyzoon being among the few survivors.

This new lake was the Lake Mœris of the ancients, and must have occupied an enormous area; Beadnell has estimated the latter to have been about 2250 square kilometres, or about ten times the area of the present Lake Qurun. The small size of the lake at the present day is due partly to evaporation, but chiefly to the reclamation of the land carried out during historic times by the different rulers of Egypt since the twelth dynasty.

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EXPLANATION OF PLATES 22 AND 23,

Illustrating Mr. Boulenger's paper on "Mœrisia lyonsi."

EXPLANATION OF LETTERING.

Cord. Stem of Cordylophora. *Ect.* Ectoderm. *End.* Endoderm. *End. Div.* Endoderm of the stomach diverticulum. *End. L.* Endoderm lamella. *E. U. Ep.* Ex-umbrella epithelium of the medusa. *Hydr.* Hydranth. *Hyp.* Hypostome. *Lat. B*^{1}. Lateral bud. *Lat. B*^{2}. Lateral bud at a later stage. *Med.* Medusæ in various stages of development. *Mes.* Mesogloea. *Nem.* Nematocyst. *Per.* Perisarcal investment. *R. Can.* Radial canal of the medusa. *R. Can. End.* Endoderm of radial canal. *Strob. B.* Buds formed by strobilisation. *S. U. Ep.* Sub-umbrella epithelium. *Tent.* Tentacles of the hydranth. *Tent. Cav.* Internal cavity of the tentacle. *Tent. L. B.* Tentacles of the lateral bud. *Test.* Testis.

FIG. 1.—The tangled colony of the hydroid growing on Cordylophora (*Cord.*). × 2.

FIG. 2.—A young colony to show the hydrorhiza with its perisarcal investment, the latter extending on to the proximal part of the branches and their growing points. The larger of the two hydranths bears an asexual bud. × 30.

FIG. 3.—A young medusa with contracted tentacles; the stomach is provided with four per-radial pouches. × 10.

FIG. 4.—An adult medusa with extended tentacles; the diverticula of the stomach extend a considerable distance down the sub-umbrellar surface. × 10.

FIG. 5.—Outline sketch of a hydranth bearing a lateral bud; the latter has become of very irregular shape, and has given rise to a small colony without becoming detached. Note the two tentacles on the bud. × 30.

FIG. 6.—Similar to the previous figure. The lateral bud has grown to a considerable size, and has given rise to a large hydranth without forming a hydrorhiza. The hydranth bears several developing medusæ and a small lateral bud with two tentacles. × 30.

FIG. 7.—Outline sketch to illustrate the formation of buds by transverse fission. A process of strobilisation at the base of a hydranth has given rise to a number of nearly spherical buds. × 30.

FIG. 8.—Transverse section of a tentacle from a hydranth. Seven large endoderm cells are seen surrounding a small but distinct cavity. × about 200.

Fig. 9.—The three types of nematocysts:—*a*. The large oval form in the unexploded condition. *b*. The same with the thread everted, only two of the three large barbs can be seen. *c*. The cylindrical nematocyst with spirally coiled thread. *d*. The pip-shaped form, unexploded, to show thread coiled round the transverse axis. *e*. The same with thread everted. Zeiss obj. F, oc. 2.

Fig. 10.—Transverse section through a stomach diverticulum and part of the umbrella of an adult medusa. The cavities of the diverticulum and radial canal are continuous, but the endodermal linings of the two structures are quite distinct. × about 60.

Fig. 11.—Similar to Fig. 10, but the section is cut through the distal extremity of the diverticulum where its cavity is separate from that of the radial canal.

The Distribution and Classification of the Onychophora.

By

Adam Sedgwick, M.A., F.R.S.,

Professor of Zoology and Comparative Anatomy in the University of Cambridge.

With 13 Figures.

The genus Peripatus, so far as adult conformation is concerned, is a very homogeneous one. It was pointed out by me in 1888 that the species from the same part of the world resembled each other more closely than they do species from other regions, and that the species in this way fall into discontinuous groups which were defined in the monograph then published. Since the publication of that monograph, specimens have been recorded from two other regions, viz. New Britain and Equatorial Africa, and the species established on these specimens completely conform to the above generalisation. So that now there are six discontinuous groups of species of Peripatus all capable of precise definition. They are (1) the South African group; (2) the Australasian group; (3) the Peripatus from New Britain; (4) those from the Neotropical Region; (5) the Peripatus from the Congo; and (6) the four species from Malaya. To these must be added a seventh group formed by the Chilian Peripatus, P. blainvillei, which, as Bouvier has shown, is quite distinct from the other neotropical species, and from all the species found in other regions. The remarkable point about these groups is that they are sharply marked off from one another, and, though there is the usual interdigitation of char-

acters, there is not the slightest difficulty in assigning all of the known species to their groups. When, in 1888, I recognised the existence of this grouping, I carefully considered the desirability of establishing them as distinct genera or subgenera, but I came to the conclusion that it would be rash to do so, partly because it was by no means certain that they would stand the test of later discoveries, and partly because the known species were so small in number and so easy to handle from a classificatory point of view, that there did not appear to be any advantage to be gained by so doing.

It is now twenty years since the publication of my monograph, and it is of interest to inquire to what extent later discoveries have confirmed or disproved the conclusions I then came to. The present is a particularly appropriate time for doing this, on account of the recent publication of Professor Bouvier's fine monograph on the genus. In this important work M. Bouvier has accurately recorded the characters of a considerable number of new species; he has defined with precision some species of which very little, sometimes nothing, was known; he has added greatly to our knowledge of the anatomy of the genus; and lastly, he has established the existence of two new primary localities for the group. By primary localities I mean localities in which all the species possess a set of characters which differentiate them from any of the groups of species previously recognised. These are the Congo and Chili. It is true that Bouvier associates these two newly defined species with certain of the older groups; he associates the Congo Peripatus with the Neotropical group, and the Chilian species with a section of the South African, but I do not agree with him in this, and it is the object of this paper to inquire (1) whether he is justified in making this association, and (2) whether the creation of genera and the association of them into families and subfamilies is in the interest of zoological science at the present time. Before doing this I may briefly recall the other important systematic work which has been done on the genus since 1888. In 1898 Willey described a new species which

he discovered in New Britain, and he showed that it possesses characters peculiar to itself which sharply differentiate it from all other known species. In 1901 Evans described three new species which he had found in the Malay Peninsula, and he showed that they possessed some peculiar features which are also presented by the Sumatran species, and are not found in any of the other known species. He thus established the reality of the Sumatran species, which I had been obliged to leave doubtful, and definitely established a new group of species from the Malayan Region distinct from the species of the other great geographical groups.

Purcell (1899) made a thorough and most valuable revision of the South African species, and discovered that one of them differs from the rest in points which seemed to justify the creation of a special genus Opisthopatus for its reception. In addition to this work, important observations were made upon the Australasian species by Fletcher and by Dendy, the last of whom discovered that some of the species from this region are normally oviparous; by Miss Sheldon on the development of a New Zealand species; by Sclater on the development of a Neotropical species; by Willey and Evans on the development of the New Britain and Malayan species respectively. With this work, however, I am not so much concerned here, excepting in so far as it adds to our knowledge of the remarkable variety in the structure of the ovum and early development in the genus.

In 1894 Pocock[1] definitely exalted three of my specific groups to generic rank, and named them: Peripatus (the American species), Peripatopsis (the South African), and Peripatoides (the Australasian). This example was followed, and the result is that we now have not only seven genera, viz. Peripatus, Peripatopsis, Peripatoides, Paraperipatus (Melanesia), Eoperipatus (Malaya), Ooperipatus (Australasia), Opisthopatus (South Africa),

[1] R. J. Pocock, "Contribution to our Knowledge of the Arthropod Fauna of the West Indies: II. Malacopoda or Prototracheata," 'Journ. Linn. Soc.' (Zool.), xxiv, 1894, p. 518.

and possibly Mesoperipatus (Congo), but long discussions as to which of them are valid and how they should be grouped in families and subfamilies. It has led, in other words, to hair-splitting and prolixity. But it has done more than this: it has led one investigator, who, by his admirable work, had definitely established the existence of a group of species (the Malayan) which I had left doubtful, to assert that my grouping of the species has "no foundation in fact," and that it had been "effectually disposed of"; and it has led workers at our genus to ignore the real problem at issue, and to substitute for it meaningless discussions as to the primitiveness of characters and phylogenetic derivation of species.

Now all this, and the very inappropriate names which have been given to some of the genera, would have been avoided if a little patience had been exercised and we had been allowed to await what future work would bring. To ascertain that we need only turn to the important facts which have been collected by the recent admirable workers in the subject—to the memoirs of Willey, Purcell, Evans, and Bouvier. These observers have clearly shown, though some of them would be far from admitting it, that the geographical grouping of the species is a perfectly sound one, and that no subordination of these into families or subfamilies is, in the present state of knowledge, either admissible or desirable. Whether it is desirable to coin generic names for these groups seems to me a matter of small importance. The important point is to recognise the fact that they exist, and to make the utmost possible use of it in our attempts to solve the great problem of species.

Personally I think it premature, unnecessary, and inconvenient to establish genera at the present moment. At the same time, it is convenient to designate each of the seven groups by a name which definitely connects it with the locality. The names which I venture to suggest are shown in the following list:

(1) The species of the neotropical region except Chili—**Neo-Peripatus.**
(2) The species from tropical Africa—**Congo-Peripatus.**
(3) ,, ,, Malaya—**Eo-Peripatus.**
(4) ,, ,, South Africa—**Capo-Peripatus.**
(5) ,, ,, New Britain—**Melano-Peripatus.**
(6) ,, ,, Australasia—**Austro-Peripatus.**
(7) ,, ,, Chili—**Chilio-Peripatus.**

I shall now attempt to show that the species of each of these groups are distinct from those of the others, and that if generic names are to be given, they must follow the lines of geographical cleavage. M. Bouvier does not accept them all. He associates Congo-Peripatus with Neo-Peripatus, and Chilio-Peripatus with that section of Capo-Peripatus, to which Purcell has given the generic name of Opisthopatus. But, as I shall endeavour to show in the sequel, the genus Opisthopatus cannot be maintained, and the Congo-Peripatus and Chilio-Peripatus are as distinct as any of the other groups.

I will begin by examining each group in detail, both from a morphological and distributional point of view, and then we shall be in a position to institute a detailed comparison between them.

NEO-PERIPATUS.—Peripatus is generally distributed in the neotropical region from Rio de Janiero in the south to Mexico in the north, and it is found in many of the West Indian Islands. West of the Andes its southern limit appears to be Bolivia. All the species in this area belong to the group Neo-Peripatus. A single species (P. blainvillei) is known from Chili (Chiloe and near Villa Rica), but this Bouvier has shown to belong to a distinct type. The characters of Neo-Peripatus, of which twenty-nine species are known, are as follows:

1. The number of legs (twenty-three to forty-three pairs) is variable in the same species.

2. Inner jaw with a diastema and a saw of denticles (Fig. 1).

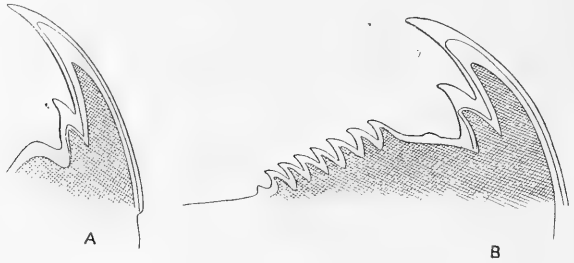


FIG. 1.—*A* outer, *B* inner blade of jaw of P. Sedgwicki.[1] (After Sedgwick.)

3. Legs with four to seven spinous pads (Fig. 2).

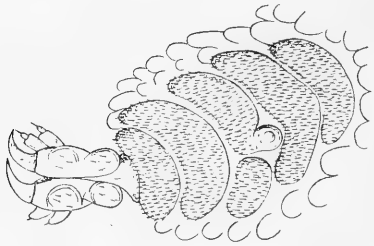


FIG. 2.—P. ecuadorensis Bouv., female. Ventral view of fourth leg, showing the pads, the position of the nephridial opening, and the pedal papillæ. (After Bouvier.)

4. Nephridial openings of legs four and five on the proximal side of the fourth pad either attached to it or separate from it.

5. Feet with three (Caribbean species), or from four to seven (Andean, Fig. 2), distal papillæ.

6. The genital opening is between the legs of the penultimate pair.

7. Oviduct is provided with a receptaculum seminis, which contains spermatozoa, and has two ducts (Fig. 3).

[1] This is the Venezuelan species referred to in the footnote on p. 395.

8. Oviduct is provided with a receptaculum ovorum (Fig. 3).

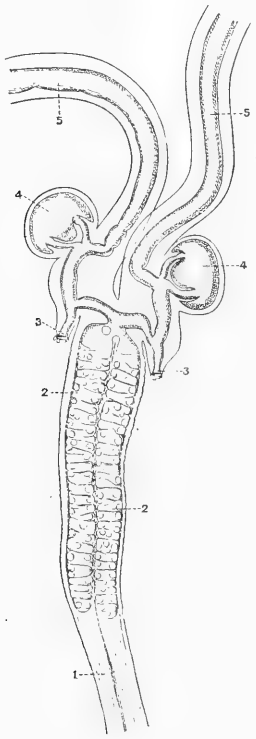


Fig. 3.—Female generative organs of P. trinidadensis. (After Gaffron.) *1*. Funiculus. *2*. Ovary. *3*. Duct or funnel of the receptaculum ovorum; the latter is not shown. *4*. Receptaculum seminis with its two ducts. *5*. Anterior end of uterus.

9. The oviducts are united at the ovary (Fig. 3).

10. The ovary is endogenous, i. e. the wall of the ovarian tube is thick, and the ova lie in the thickness of the epithelium (Fig. 4). This character was first called attention to by Willey (1898), who contrasts it with the condition found in Capo- and Austro-Peripatus, etc., in which the wall of the ovary is thin, and the maturing ova do not retain their

epithelial position, but cause the ovary to project in the form of follicles into the body-cavity (exogenous ovary, Fig. 4).

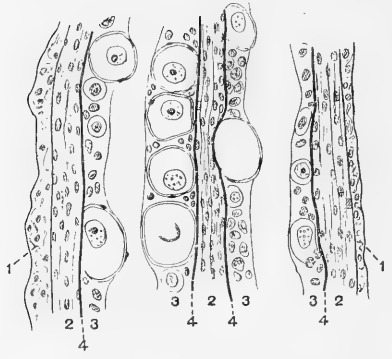
11. Ova minute, ·04 mm. in diameter.

12. Embryo provided in its young stages with a trophic vesicle, within which it lies.

13. Uterine embryos of all ages and born all the year round.

14. Unpaired part of vas deferens long and complicated.

Fig. 4. Fig. 5.



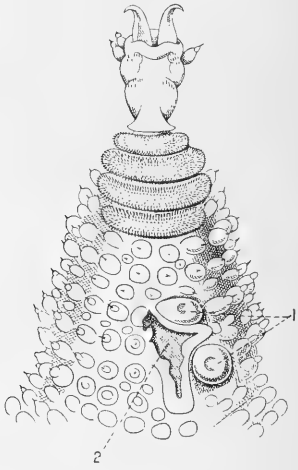


Fig. 4.—Horizontal section through the ovarian tubes of P. trinidadensis. (After Gaffron.) *1.* Peritoneum traversed by tracheæ. *2.* Tunica muscularis. *3.* Germinal epithelium. *4.* Tunica propria.

Fig. 5.—Ventral view of one of the posterior legs of a male P. Sedgwicki[1] Bouv. (After Sedgwick.) *1.* Papillæ of crural glands. *2.* Coxal organ. There are two papillæ on the anterior side of the distal part of the foot and one on the posterior.

15. Spermatophores elongated and with a thick case; often only one is present.

16. Skin pigment brownish, extracted by alcohol. The pigment appears to be dissolved out by the alcohol, which becomes tinged with brown, but the colour soon fades.

[1] See footnote on p. 384.

17. Legs with well-developed coxal organs (Fig. 5). There are furrows on the ventral side of the bases of the legs provided with tumid lips and lined by a smooth, non-tuberculate epithelium.

18. Crural glands in many legs in the males opening on papillæ (Fig. 5).

19. The accessory glands of the male open separately at the sides of the anus.

Fig. 6.—Fourth leg of a female P. Tholloni, ventral view. (After Bouvier.)

CONGO-PERIPATUS.—A single species, P. Tholloni, has been described by M. Bouvier from the French Congo. They were found among leaves on vegetable humus round the trunk of an Elais guineensis at Ngômô in Ogôoué. Only a few specimens have been found, and it is possible that other species will be discovered. It is quite distinct from the Capo-Peripatus, and must be regarded as constituting a distinct type. Its characters are as follows:

1. Number of legs (twenty-four to twenty-seven pairs) is variable in the same species.

2. Inner jaw with a diastema and a saw of denticles.

3. Legs with three spinous pads (Fig. 6).

4. Nephridial openings of legs four and five on the proximal side of the third pad (Fig. 6) and attached to it.

5. Feet with three distal papillæ (two in front and one behind).

6. Genital openings between the legs of the penultimate pair.

7. Oviduct is provided with a receptaculum seminis. It is not known whether it contains spermatozoa or has two ducts.

8. There is a receptaculum ovorum smaller than in Neo-Peripatus.

9. The oviducts and ovaries are entirely separate from one another.

10. It is not clear from those writings of Bouvier which are accessible to me (1907), whether the ovary is endogenous or exogenous. I gather that it is endogenous.

11. Size of ripe ova not determined, not less than ·06 mm. (Bouvier, '07, p. 345).

12. Young embryonic stages not known.

13. Uterine embryos differ much in age. Those near the uterine opening collect into a large uterine dilatation.

14. Unpaired part of vas deferens of great length.

15. Spermatophores unknown.

16. Skin pigment brownish, extracted by alcohol.

17. Legs with well-developed coxal organs.

18. Crural glands in the two pairs of pre-genital legs in male (two pairs on each leg) opening on papillæ.

19. The accessory glands of the male open in front of the anus in a common furrow.

Bouvier, in commenting on this species ('07, p. 336) deprecates giving it a generic name on the ground that we do not know what the future discoveries in equatorial Africa have in store for us; precisely the same reason, be it observed, that actuated me in declining to name my four groups. It would be interesting to know M. Bouvier's reasons for adopting generic rank for those species while declining it for his own.

This species undoubtedly has, at first sight, a strong look of Neo-Peripatus, but the characters 3 + 5, 9, and 13, in which it appears to be unique, are sufficient to separate it; and it must not be forgotten that we are ignorant of the nature of its egg and early development. In 18 and 19 it seems to resemble only Eo-Peripatus.

EO-PERIPATUS.—Four species are known in Malaya, three from the States of Jalor and Kelantan in the Peninsula (Evans), and one from Sumatra (Horst, Evans). The characters are as follows:

1. Number of legs (23—25 pairs) usually variable in the same species.

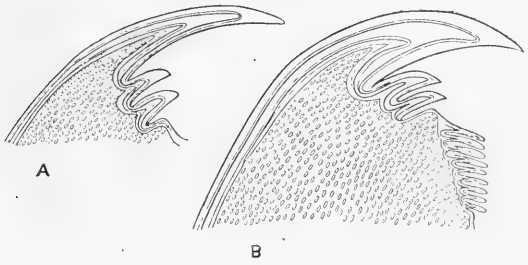


FIG. 7.—*A* outer, *B* inner jaw of P. Weldoni. (After Evans.)

2. Inner jaw with a diastema and a saw of denticles (Fig. 7).

3. Legs with four spinous pads.

4. Nephridial openings of legs four and five either in the proximal pad (Weldoni, Sumatranus) or proximal to it (Horsti, fig. 8).

5. Feet with two distal papillæ, one on the anterior and one on the posterior side.

6. Genital opening between the legs of the penultimate pair.

7. Receptacula seminis, with two ducts opening into the oviducts, are present.

8. Receptacula ovorum are present opening into the oviducts close to the ovary; no eggs have been found in them.

9. The oviducts are united at the ovary.

9*a*. The ovaries are completely fused, and contain one spacious cavity, and are attached to the floor of the pericardium by an extensive surface.

10. The ovary is exogenous, i. e. it is studded with follicles in which the maturing ova lie.

11. The ova are large and heavily charged with food-yolk. They measure about 1 mm. in their longest diameter.

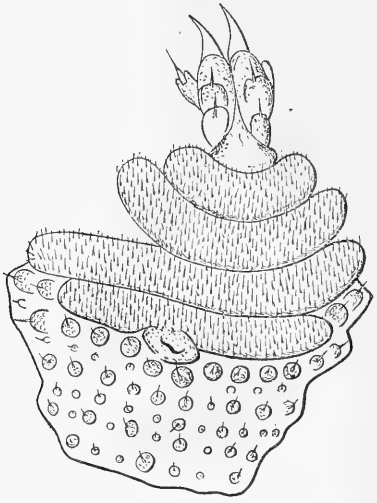


Fig. 8.—Ventral view of fifth leg of female P. Horsti. (After Bouvier.)

12. Embryo without a trophic vesicle.

13. Uterine embryos of all ages.

14. Unpaired part of vas deferens nearly as long as that of Neo-Peripatus.

15. A single long spermatophore without a horny coat, but with a horny cap at its front end.

16. Skin pigment brownish; it is not stated that it is affected by alcohol.

17. Legs with well-developed coxal organs.

18. Crural glands in the male in the two pairs of legs preceding the genital opening; there are two pairs in each leg; they open into the groove of the coxal organ, and are without papillæ.

19. The accessory glands of the male open between the legs of the last pair by a common opening.

This is an exceedingly interesting group of species, for it shows, together with several peculiar features, viz. 3 + 4, 5, 9*a*, an important character only found elsewhere in Austro-Peripatus, viz. 11; and in the form of its jaws, in the number of its spinous pads, the position of the genital opening, the presence of a receptacula ovorum, it approximates markedly to Neo-Peripatus. But it is removed from Neo-Peripatus by its exogenous ovary, as well as by its large ova. It appears to hold 14 and 15 in common with Neo-, Congo-, and Austro-Peripatus. On the whole this group of species is as distinct as any, and is especially interesting as showing a mingling of characters which are found elsewhere only in Neo-Peripatus or in Austro-Peripatus. On the other hand it is worthy of remark that it shows nothing in exclusive community with Capo-Peripatus.

MELANO-PERIPATUS.—In 1897 Peripatus was discovered by Dr. A. Willey in New Britain. He wrote a full description of its anatomy and development, and named it P. novæ-britanniæ. Its characters are as follows:

1. The number of legs (22—24 pairs) is variable in the species.

2. The outer jaw is without a minor tooth, and the inner jaw has no diastema or saw.

3. Legs with three spinous pads.

4. Nephridial opening of legs 4 and 5 are on the proximal pad.

5. Feet with three distal papillæ, one of which is anterior, one dorsal sometimes inclined to the anterior side, and one posterior (Fig. 9).

6. Genital opening subterminal behind the legs of the last pair.

7. Oviduct with a receptaculum seminis with two ducts.

8. Receptacula ovorum absent.

9. The oviducts are united at the ovary.

10. The ovary is exogenous.

11. Ova of medium size (·1 mm. in longest diameter) with little yolk.

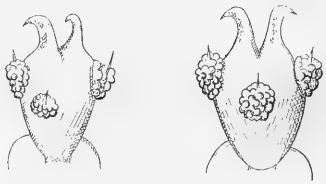


Fig. 9.—Dorsal views of feet of P. novæ-britanniæ, showing the primary papillæ. In *a* the dorsal papilla is inclined to the anterior papilla; in *b* it is median. (After Willey.)

12. Embryo in its young stages provided with a large dorsal appendage, consisting of ectoderm and endoderm, and called by Willey the trophic vesicle.

13. Uterine embryos of all ages in the same uterus.

14. Unpaired part of vas deferens, very short, almost obsolete.

15. Spermatophores absent.

16. Skin-pigment black, apparently not affected by spirit.

17. Legs without well-developed coxal organs.

18. Crural glands absent.

19. Accessory glands of the male open medianly and dorsally near the hind one.

This species, which we should expect from its locality to resemble Austro-Peripatus, presents seven peculiar features, 6, 11, 12, 14, 15, 19, of which 6, 11, 14, and 19 are important morphologically. By having a receptaculum seminis with two ducts and no receptacula ovorum it presents a combination of characters found elsewhere only in

Austro-Peripatus. The skin-pigment appears to be peculiar, but in not being affected by spirit it approaches Capo- and Austro-Peripatus; and the same may be said about the absence of well-developed coxal organs, and the form of the jaws. Its affinity would appear to be closer to Austro- and Capo-Peripatus than to Neo-Peripatus, but it differs from Austro-Peripatus in the variability of its leg number (1) and in its ovum. On the other hand it has no crural glands, a feature which is found elsewhere only in Austro-Peripatus. The form of its ovum (11) and its trophic vesicle (12), though peculiar characters, are nearer the corresponding structures in Capo-Peripatus, as also is the structure of its vas deferens (14). On account of the number of its peculiar features we have no hesitation in establishing it as a distinct group, but we think it is considerably nearer to Capo-Peripatus than to Austro-Peripatus, from which it is distinguished by the very important characters of its ovum as well as by its leg-number.

CAPO-PERIPATUS.—Peripatus is found in Natal and Cape Colony. It is represented by seven well-established species (Purcell, 1899), which exhibit the following characters:

1. The number of legs (16—25 pairs) is variable when the number of pregenital legs exceeds 19 pairs.

2. Outer jaw with one minor tooth; inner jaw without diastema or saw.

3. Legs with three spinous pads.

4. Nephridial openings of legs 4 and 5 on the proximal pad.

5. Feet with three distal papillæ, two anterior and one posterior, except in one species, cinctipes, in which one of the anterior papillæ is dorsal.

5*a*. Feet with two papillæ at the base of the foot (Fig. 10), except in one species, cinctipes.

6. Genital opening between the legs of the last pair which

show a tendency to reduction, and are sometimes obsolete (P. capensis).

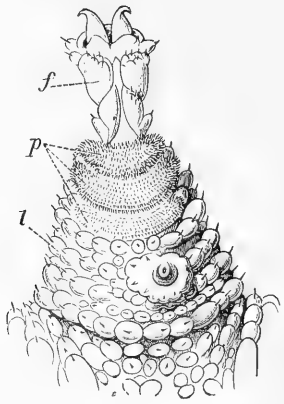


Fig. 10.—Ventral view of last leg of a male of P. capensis. (After Sedgwick.) *f.* Foot. *l.* Leg. *p.* Spiniferous pads. The papillæ on the proximal part of this leg is characteristic of the male of this species.

7. Receptaculum seminis absent, except in cinctipes, in which there is said to be a minute trace of one. It is, however, caused by a loop of the epithelial tube (Fig. 11).

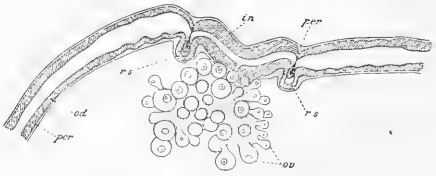


Fig. 11.—Ovary and oviduct of P. cinctipes, showing the minute receptaculum seminis, *r.s.* (After Purcell.) *in.* Thick wall of infundibular part of the oviduct. *od.* Thinner wall of oviduct. *ov.* ova. *per.* Peritoneum and muscles of oviduct.

8. Receptacula ovorum absent.
9. Oviducts united at ovary (Fig. 11).
10. The ovary is exogenous.
11. Ova comparatively large, with but little yolk (·56 mm.

in greatest diameter in P. capensis, in P. Balfouri ·4 mm.; in P. cinctipes it is smaller, probably about ·2 mm., but its length is not given by Purcell).

12. Embryos usually without trophic vesicles, but Bouvier states that there is a tendency to a dorsal trophic vesicle like that of Melano-Peripatus in two species (P. Sedgwicki[1] and Moseleyi).

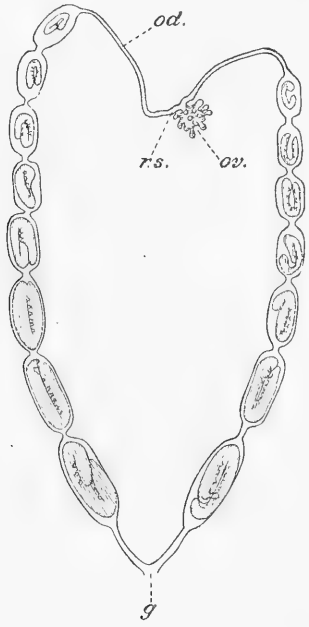


Fig. 12.—Female generative organs of P. cinctipes. (After Purcell.) *ov.* Ovary. *rs.* Receptaculum seminis. *od.* Oviduct. *g.* Genital opening.

13. Uterine embryos of nearly the same age. They differ most in P. cinctipes (Fig. 12), in which, however, the difference is not very great.

14. Unpaired part of vas deferens short.

[1] So named by Purcell. Bouvier has applied the same name to a species of Neo-Peripatus found in Venezuela (this footnote is referred to on p. 384).

15. Spermatophores small, oval, with a thin coat.

16. Skin pigment blue, green, or black, not affected by spirit. A certain amount of brown or orange is often present, especially on the ventral surface.

17. Coxal organs variable; usually not well developed. In P. Sedgwicki and Moseleyi they are moderately developed, and in P. cinctipes well developed.

18. Crural glands present in most legs of both sexes, except possibly in P. cinctipes, in which they have not been found by Purcell in the females.

19. The accessory glands of the male open into the terminal part of the vas deferens, except in P. cinctipes, in which they open separately between the anus and genital opening.

The South African group of species presents a greater variation of structure in the selected characters than any of the other groups.

The variable characters are:—(1) The legs on each side of the genital opening, No. 6. (2) The coxal organs, No. 17. (3) The size of the ova, No. 11. (4) The presence of a trophic vesicle. These vary throughout the group. In addition to these we find in P. cinctipes variability in the pedal papillæ, a variability similar to that found in the same character in Neo-Peripatus. Moreover, this species is said to possess a minute receptaculum seminis found in no other species of the group, and a variation in No. 19. P. cinctipes, on account of its peculiarity in these three characters, Nos. 5, 7, 19, has been established by Purcell as a special genus, Opisthopatus, which has been accepted by Bouvier. I find myself quite unable to admit that the characters mentioned are sufficient to justify the separation of P. cinctipes from the rest of the group. The differences are not greater than those which separate the Andean from the Caribbean species of Neo-Peripatus, and no one has proposed to separate these from one another.

The assemblage of characters which leads me to assert the homogeneity and distinctness of the group are those numbered

2, 3, 4, 6, 7, 11, 12, 13, 14, 15, 16, 17, and 18. Of these the most important, and not found in any other group, are 7, 11, and 13 (the receptaculum seminis of P. cinctipes is so very small and simple that it hardly affects the point). But, although I assert the distinctness of the group, it must be confessed that it approaches Melano-Peripatus more closely, perhaps, than do any other two groups of species, not even excepting Neo-Peripatus and Congo-Peripatus. This is especially shown by the form of the jaws and the nature of the ovum, No. 11; through the species cinctipes in the arrangement of the distal pedal papillæ, No. 5; through the species Capensis in the absence of genital legs, No. 6; through the species Sedgwicki and Moseleyi in the tendency towards the presence of a trophic vesicle, No. 12; through most of the Cape species in the absence of well developed coxal organs, No. 17; and in the shortness of the unpaired part of the vas deferens. But the distinctness of Melano-Peripatus cannot be impugned, having regard to its characters 6, 7, 11, 12, 14, 18, and 19.

AUSTRO-PERIPATUS.—Peripatus is known from both Eastern and Western Australia, from Tasmania and from New Zealand. It is interesting to note that it has not so far been found in New Guinea. All the species known, of which there are eight, belong to the group Austro-Peripatus (Peripatoides of recent authors). The characters are as follows:

1. The number of legs (14 to 16 pairs) is constant in the same species.

2. The outer jaw is either without minor teeth or with one or more minor teeth; the inner jaw is without a diastema or a saw.

3. The legs have three spinous pads.

4. Nephridial openings of legs 4 and 5 on the proximal pad.

5. Feet with three distal papillæ (one anterior, one dorsal,

and one posterior), except in P. suteri, in which there may be three or four.

6. The genital opening is between the legs of the last pair, which are normally developed.

7. The oviduct is provided with a receptaculum seminis with two ducts.

8. Receptacula ovorum absent.

9. The oviducts are united at the ovary.

10. The ovary is exogenous.

11. The ova are very large (from 1·5 to 2 mm. in longest diameter) and heavily yolked.

11*a*. In some species the opening of the vagina is at the end of a long ovipositor (viridimaculatus, insignis, Leuckarti, oviparus) and the eggs are probably laid. P. oviparus and viridimaculatus are certainly oviparous, and their eggs have sculptured shells.

12. Nothing corresponding to a trophic vesicle.

13. Uterine embryos of markedly different ages or of about the same age.

14. Unpaired part of vas deferens long and complicated.

15. Spermatophore single and elongated, with a thick case.

16. Skin pigment mainly black, blue, green, or brown, unaffected or but slightly affected by spirit.

17. Legs without well-developed coxal organs.

18. Crural glands present in some species (Suteri, novæ-zealandiæ), absent in others. When present, in the males only.

19. Accessory glands of the male opening separately between the anus and genital opening; in some species the openings are far apart, in others (P. Leuckarti) close together between the genital opening and the anus.

On the whole, this group of species, which ranges over the whole of Australia, Tasmania, and New Zealand, presents but little variation. There is a little variation in the outer blade of the jaw, in the pedal papillæ, in the relative ages of embryos in the same uterus, and in the crural glands. Also in three of the species the oviduct opens at the end of a

papilla—the ovipositor. This character is associated in two of the three species (and possibly in the third) with an oviparous habit and a sculptured egg-shell. It has been proposed to segregate these three species in a special genus, Ooperipatus, but, having regard to the fact that all Austro-Peripatus approach the oviparous condition, some even abnormally extruding eggs, this character of oviparity cannot be regarded as sufficiently important to confer generic rank. This view is still further emphasised by Bouvier, who points out that to adopt it will create confusion in the arrangement of the other species of the group, the oviparous forms not being monophyletic.

The only character absolutely peculiar to the group is No. 1. The other characters are distributed fairly impartially in most of the other great groups. Thus No. 6 is found in some of the Capo-Peripatus and then only imperfectly, and the colour also takes after that of the South African forms. The presence of well-developed receptacula seminis without receptaculum ovorum is found elsewhere only in Melano-Peripatus; and the absence of well-developed coxal organs and the arrangement of the pedal papillæ occur again only in Melano-Peripatus, Chilio-Peripatus, and some species of Capo-Peripatus. The character and size of the ova are found elsewhere only in Eo-Peripatus, and the length of the unpaired part of the vas deferens and nature of the spermatophore only in Neo-, Congo-, and Eo-Peripatus. From this summary it is doubtful, to say the least of it, if Austro-Peripatus shows any special affinity to any other group of species. At first sight one might be inclined to assert an approach to Melano- and Capo-Peripatus, but having regard to the important characters Nos. 1, 11, and 14 this view can hardly be maintained.

CHILIO-PERIPATUS.—One species of Peripatus has for long been known from Chili, but it is only comparatively recently that its characters have been made known to us by Bouvier. It occurs far to the south of any Neo-Peripatus,

and is entirely distinct from that group.[1] Its characters are as follows:

1. Number of legs variable (19 to 21) in the same species.

2. Outer jaw with two minor teeth, inner jaw without a diastema and saw.

3. Legs with three spinous pads.

4. Nephridial opening of legs 4 and 5 on the proximal pad.

5. Feet with three distal papillæ, one of which is dorsal.

6. Genital opening between the legs of the last pair, which are reduced in size.

7. Receptaculum seminis, if present, very much reduced, without double duct.

8. Receptaculum ovorum absent.

9. Oviducts united at the ovary.

10. The ovary is endogenous.

11. Ova small (·07 mm.), but not so small as in Neo-Peripatus.

12. The embryos are without a trophic vesicle.

13. Uterine embryos of markedly different ages, but arranged in groups of three, the embryos of each group being of the same age (Fig. 13).

14. Unpaired part of vas deferens short as in Capo-Peripatus.

14*a*. A part of the vas deferens on each side is coiled into a close spiral.

15. The spermatophores are multiple, small, and cylindrical, without a specially thick case.

16. Green or black, with reddish patches, but little affected by spirit.

17. Without well-developed coxal organs.

18. Crural glands unknown in either sex.

19. The openings of the accessory glands of the male are unknown.

[1] It is beyond the scope of this paper to deal with the geographical significance of the occurrence of a distinct specific group of Peripatus on this part of the South American Continent.

I think that there can be little doubt that an impartial consideration will assign to this species the rank of an independent group. Though it approaches by its colour, and by the characters of its jaws, legs, and feet, Capo-, Austro-, and Melano-Peripatus; it differs absolutely from those groups by its endogenous ovary, found elsewhere only in Neo-Peripatus, by the small size of its ova, and by the important characters 13 and 14*a*. By its feet it is more

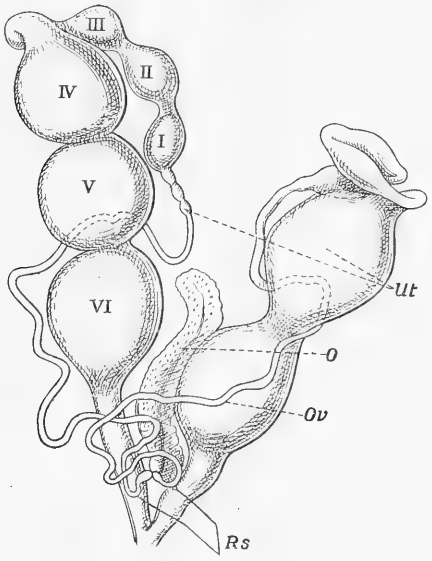


Fig. 13.—Ovary and gravid uterus of P. Blainvillei. (After Bouvier.) *Rs*. Supposed receptaculum seminis. *O*. Ovary. *Ov*. Oviduct. *Ut*. Uterus. I, II, III. Embryos of one group. IV, V, VI. Embryos of the next group.

especially approached to Austro-Peripatus, by the unpaired part of its vas deferens to Capo-Peripatus. It is undoubtedly more closely allied to the Austro- and Capo-Peripatus than to the Neo-Peripatus, but its ova approach those of Neo-Peripatus more closely than do those of any other form, and it has an endogenous ovary. We get, therefore, here again the same intermingling of characters of

different species-groups that we have before met with in each of the species-groups, together with a certain number of entirely peculiar features. In assigning to Chilio-Peripatus (Peripatus Blainvillei) independent rank I am at issue with Bouvier. He associates it with the South African species Peripatus cinctipes in Purcell's genus Opisthopatus. He bases this association on the character of the ovum (considerably smaller than that of cinctipes), on the arrangement of the distal pedal papillæ (found also in Melano- and Austro-Peripatus), and on the presence of a very minute receptaculum seminis. But, as stated above, the ovum is much smaller than that of cinctipes, approaching more closely to that of Neo-Peripatus, and it is doubtful if the very small dilatation called receptaculum seminis is really homologous with that structure in other species. I am quite unable, therefore, to admit the association of Blainvillei with cinctipes any more than I can admit the genus Opisthopatus itself. It seems to me clear that Bouvier, in placing it with cinctipes, has been actuated largely by his hypothetical views as to the place of origin and evolution of the genus. These views, based, as such views generally are, upon à priori considerations as to the primitiveness of certain characters and the more recent origin of others, have no real validity, and tend to obscure the important issues, upon which the facts of classification and distribution can help to throw light. The futility of this method of speculation is further emphasised by the fact that the results obtained by it vary directly according to the theoretical bias of the author. Bouvier, who regards the presence of the maximum number of structures (1907, p. 70), whether legs, segments, spinous pads, etc., as a sign of primitiveness, places Neo-Peripatus nearest a supposed original form, whereas Evans (1901*a*, p. 525), who considers a large and heavily yolked egg as an antique character, regards as the most primitive group Eo-Peripatus, which by Bouvier is regarded as the most advanced. Such diverse results may well inspire doubt as to the value of the method employed.

What, then, are the important lessons which the distribution of the species of the genus Peripatus teach us. They are, I take it, two in number:—(1) The geographical groups of species are natural zoological groups, the members of which are more closely related to each other than to those of the other groups. (2) The distinguishing specific characters are distributed in an entirely haphazard manner in the different specific groups, so that it is quite impossible to show the phylogenetic affinities of the specific groups by any tree-like arrangements.

On the first of these two points I have already said enough; enough, I hope, to convince all candid minds of its truth. On the second it is necessary to dwell a little longer. Let me take the case of Eo-Peripatus, which is associated by Bouvier with Neo-Peripatus in his family Peripatidæ. It borrows, so to speak, its large and yolked ovum from Austro-Peripatus; the number of its spinous pads from Neo-Peripatus; its inner jaw, the position of its genital opening, its receptacula ovorum, its skin pigment from Neo- and Congo-Peripatus; its receptacula seminis, the length of the unpaired part of the vas deferens, the form of the spermatophore from Congo, Neo-, and Austro-Peripatus; the opening of the accessory glands of the male from Congo-Peripatus; and lastly, the well-developed coxal organs from Congo- Neo-, and some of the Capo-Peripatus. And, in addition to this mingling of characters of other groups, it possesses the following peculiar features found in no other species: the position of the nephridial opening of legs 4 and 5, the number of distal pedal papillæ, and the complete fusion of the two ovaries (No. 9*a*).

Or again, let us take Congo-Peripatus, which is associated by Bouvier in the same genus as Neo-Peripatus. It has only borrowed one character exclusively from Neo-Peripatus, viz. the position of the nephridial openings on legs 4 and 5. It has borrowed the opening of its accessory glands and crural glands from Eo-Peripatus; its inner jaw, the position of its genital opening, its receptacula ovorum, its skin pigment from

Neo- and Eo-Peripatus; its receptaculum seminis, the length of the unpaired part of its vas deferens from Neo-, Eo-, and Austro-Peripatus; its distal pedal papilllæ from Capo-, Austro-, and Neo-Peripatus; its spinous pads from Capo-, Melano-, and Austro-Peripatus; its well developed coxal organs from Eo-, Neo-, and some Capo-Peripatus. Lastly, in the complete separation of its female generative tracts (No. 9), and the peculiar method of housing its uterine embryos (No. 13), it stands apart from all other species. Looked at in this comprehensive way, which is the only fair way, there seems to be nothing to be said for placing P. Tholloni with Neo-Peripatus.

Lastly, let us look at Peripatus cinctipes, which occurs geographically with Capo-Peripatus, and consider whether it ought to be separated from this group and placed in a special genus. The series of forms found in South Africa admittedly present a greater variation in structure than any other group. Some of these variations are found throughout the group, others are confined to P. cinctipes. It will only be necessary for us to consider the latter. These are (1) a difference in the number and position of the pedal papillæ, (2) the presence of a minute receptaculum seminis, (3) a difference in the method of opening of the accessory glands of the male. With regard to (1) the distal papillæ vary from the type less than do those of the Andean species of Neo-Peripatus amongst one another, and a very similar variation is presented by different individuals of the single species of Melano-Peripatus. But this is not the only difference from the type; the basal pedal papillæ, so characteristic of the Capo-Peripatus in general, are absent. This, though important, is not sufficient to justify the establishment of the genus Opisthopatus. Nor are the other two; for the receptaculum seminis is different to the usual form of that organ, and so small that its homology is doubtful, and it may easily have been overlooked in other species, and the difference in the openings of the accessory glands is parallelled in Austro-Peripatus. The absence of the basal pedal papillæ

not being sufficient in itself to justify the establishment of a genus (it is quite possible that further research on Capo-Peripatus may disclose other species in which they are absent), I am led to reject the genus Opisthopatus.

It would be tedious to continue this detailed examination. The reader can easily do it for himself, and if he does so he will, I think, convince himself of the truth of my two main contentions: (1) that the geographical groups are natural groups, (2) that the distinguishing specific characters are distributed in an entirely haphazard manner amongst them. The conclusions which I draw from these facts (as I venture to regard them) are two in number. First I infer that the present species of Peripatus are derived from a single widely ranging species roughly extending within the limits of the present distribution; and secondly, that this species was highly variable, including within the range of its variation all the different characters at present presented by the whole genus.

Of these two inferences the first will generally be conceded to have some possibility of truth. The second is not so obvious. It is one which I have long held as a principle which explains many difficulties and anomalies in classification. I base it on the significant intermixture of characters, which is presented not only by the genus Peripatus but by many groups of the animal kingdom; an intermixture of characters which on the ordinary views of the theory of evolution are quite incompatible with one another; an intermixture of characters, which, by all the canons of ordinary morphological criticism, are primitive, with characters which are specialised. I have called attention to the phenomenon more than once in my work on 'Zoology.' For instance, in the second volume, p. 618, in dealing with the relationship of the main groups of the Carnivora I say, "What we have here is merely an example of the principle to which we have so often before called attention in this work, that the more closely any given group of animals is studied, the more complex are the mutual relations between its different mem-

bers found to be," and I refer the reader to the case of the Nudibranchiata in vol. i, p. 410, which prominently illustrates the same principle. I might also refer to the case of fishes (Chimæra and the Dipnoi) as remarkable instances of the same phenomenon.

The case imagined for Peripatus is very similar to that of the human race, which consists of an almost continuous, interbreeding and variable species. The Australian may be said to bear the same relation to the negro—lacking the frizzy hair, as Eo-Peripatus does to Neo-Peripatus—lacking the small ovum.

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A few Observations on the Encystation of Actinosphærium eichhorni under different conditions of Temperature.

By
Doris L. Mackinnon, B.Sc.,
Carnegie Scholar, 1906–1907.

With Plate 24 and 1 Text-figure.

Previous to Richard Hertwig's exact study "Über Kernteilung, Richtungs-Körperbildung und Befruchtung von Actinosphaerium eichhorni" (1898), our knowledge of the life-cycle in this heliozoon was still very incomplete. Hertwig's work laid clear for the first time the complicated series of changes that take place within the Actinosphærium cyst, and his subsequent work, Über Physiologiche Degeneration bei Actinosphaerium eichhorni" (1904), threw further light on the conditions affecting the life-processes in this organism, both free-swimming and encysted.

In the last few years cytologists have found in Actinosphærium a highly favourable object for experimental cell-study. Mr. Geoffrey Smith, of New College, Oxford, working in Munich in 1902, made interesting comparisons between cultures brought to encyst at different temperatures. While in Professor Hertwig's laboratory in Munich, early in 1907, I undertook, at his suggestion, a series of similar experiments, to determine, if possible more exactly than previous results

suggested, the effects of high and low temperature on the nucleo-cytoplasmic proportions in the Actinosphærium cyst.

Briefly stated, the normal course of encystation, as observed by Hertwig, is as follows:—Encysting Actinosphæria withdraw their pseudopodia, adhere to the substratum, and take on an opaque, greyish appearance. This is the stage of the mother-cyst; during it a silica coat begins to form, and about 95 per cent. of the nuclei present in the unencysted state disappear. The mother-cyst divides up into a varying number of primary cysts, each containing one nucleus: that is to say, the number of the primary cysts formed by a given Actinosphærium depends on the number of nuclei remaining over from the mother-cyst reduction. Each primary cyst gives rise by division to two secondary cysts. From the nucleus of each of these, two polar bodies are given off. The secondary cysts then fuse, two and two, each pair of nuclei forming together the single nucleus of a conjugation-cyst. From this conjugation-cyst the free-swimming Actino-sphærium is finally liberated. Encystation can be artificially induced by starving Actinosphæria that have previously been feeding well.

In April I prepared starvation-cultures from fresh Actinosphærium material fetched from a pond at Possenhofen, near Munich. I chose for my purpose 90 or 100 individuals of approximately similar size, and kept half of them in closely covered watch-glasses of clean culture-water at a temperature of 10° C., the other half at 25° C. I allowed them no food, and merely from day to day removed impurities, and put in fresh water. When encystation had occurred, as happened usually within three or four days, I killed them off at the primary-cyst stage, and substituted fresh cultures. (In the beginning I kept a third line of cultures going at an intermediate temperature of 17° C. Soon, however, as the weather grew warmer, it was impossible to keep the room where the cultures stood at this low temperature, and I gave up the attempt.)

At first, though encystation took place rapidly and nor-

mally, I did not obtain satisfactory material. I either allowed the cultures to develop too far, or I killed off different, and, therefore, non-comparable, stages in the two temperatures. I also lost much good material through subsequent inexperienced handling with reagents. Owing to the thickness and opacity of the silica coat the cysts are troublesome objects to stain and clear. Finally, when I had attained some proficiency in treating my material, the cultures began to encyst abnormally. I did not succeed in preparing perfectly parallel stages from both sides of a normal culture till the beginning of June, and then from Possenhofen material fetched in on the 9th of May. After that depression set in, and subsequent cultures from the same source were again abnormal.

The material for the April cultures I named A, that used from the 10th of May till the 4th of June, B. The following table illustrates the course of B cultures:

Culture.	First day of starvation.	Fate in warmth (25° C.).	Fate in cold (10° C.).
B 1	10th May	Attempt at encystation.	Die off without encysting.
B 2	11th ,,	Satisfactory encystation.	Majority encyst, but with abnormalities.
B 3	14th ,,	15 animals encyst out of 40.	20 animals encyst out of 40.
B 4	15th ,,	19 ,, ,, ,, 45.	10 ,, ,, ,, 45.
B 5	18th ,,	7 ,, ,, ,, 23.	5 ,, ,, ,, 23.
B 6	27th ,,	12 ,, ,, ,, 25.	Attempt at encystation.
B 7	29th ,,	None encyst.	None encyst.
B 8	31st ,,	A few primary cysts formed.	8 encyst.
B 9	1st June	Satisfactory encystation.	Satisfactory encystation.
B 10	2nd ,,	Encyst abnormally.	Die off without encysting.
B 11	4th ,,	Encyst, but with some abnormalities.	Attempt at mother cysts.

That is to say, all cultures except B 9 showed some abnormality in the warmth or in the cold, and sometimes in both. For my purpose it was essential that the cysts should be normal. B 9 was the only culture where I obtained completely satisfactory final results.

Study of this table shows that during my observations Culture B underwent two periods of deep depression, when no cysts were formed. From such a depression the culture recovered till the maximum of "encystability" was reached, when it again degenerated.

Abnormality almost always showed itself first in the cold side of the culture; which suggests that a lowered temperature weakens the resistance of the organism to unfavourable internal conditions. That lowered temperature has some paralysing effect on the ordinary cell-functions is further borne out by the much greater length of time required to elapse before encystation begins in a cold culture, and by the relative slowness with which the successive stages are reached in it. The following table illustrates this :—

Culture.	First day of starvation.	Date of beginning of encystation. In the warmth.	In the cold.
B 3	14th May	15th May	20th May
B 5	18th ,,	20th ,,	24th ,,
B 6	27th ,,	29th ,,	5th June
B 8	31st ,,	1st June	4th ,,

That is to say, encystation usually sets in within forty-eight hours in the warmth, but not until four or five days later in the cold.

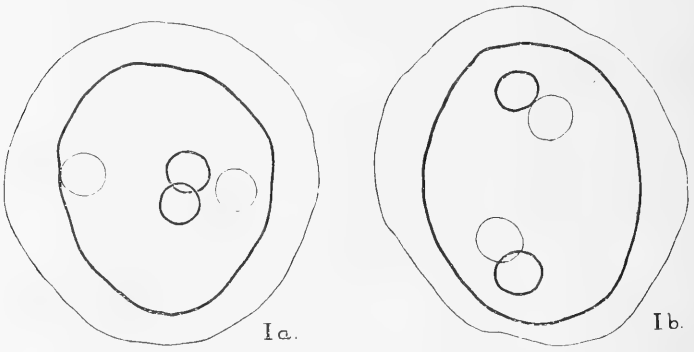
The primary cysts afforded the most favourable material for observation, as in this stage the silica-coat had not yet developed to great thickness. I fixed the primary cysts then with picro-acetic acid, stained them with borax carmine, and cleared them with clove-oil. In nearly every case in culture B 9, which was the one I used for taking measurements, the primary nuclei had already divided into secondary nuclei, though division into secondary cysts had not yet begun : frequently, however, the nuclei had become heteropolar. Successful preparation showed the cytoplasm stained a faint rose-colour, while the outlines of the nuclei, and even their finer internal structure, shone quite clearly through the silica coat. When the cysts were no longer normal, the excessive development of silica made this clear view impossible.

I examined the cysts while they were in clove oil, and took measurements of their length and breadth—these measurements I found to be very constant so long as the culture was normal. Even to the eye there was at once a quite striking difference between cysts from the warm culture and those from the cold. Those from the warm were noticeably the larger, and of more oval form, with a thin coat of silica: those from the cold were smaller, and more rounded in outline; they lay together more compactly, and showed a tendency to greater production of silica. My measurements brought out the size-difference strikingly, the average length in the warmth being 118·5 μ, and the breadth 87·5 μ, while in the cold the average length was 83·5 μ, and the breadth 77·5 μ.

I had not taken the pains to measure the Actinosphæria used for these cultures, nor had I observed whether fusion took place between two or more during the starvation period previous to encystation. I therefore cannot say that, in the compared cultures, the groups of cysts arose from exactly equally-sized individuals. Still, in a general way, I had chosen Actinosphæria of about the same size. Therefore, it is at least interesting to note that the average number of primary cysts formed by one individual was in the cold culture 5, as compared with 3 in the warm. Seven or eight cysts in a group was not uncommon in the cold, but in the warmth was rare. In the warmth sometimes only two cysts, and twice only one cyst of large dimensions were formed by one individual. Now if, as has been stated, the number of cysts formed depends on the number of nuclei remaining over from the reduction in the mother cyst, then this larger number of cysts formed at a low temperature must mean that, in the cold, a larger number of nuclei escape destruction. The smaller size of these cysts is the necessary result of their greater number, there being less cytoplasm available for each. Mr. Smith's experiments gave the same general result. ('Biometrica,' vol. ii, 1903).

To obtain measurements of the nuclei in the primary cysts, I made sections, 10 μ thick, of the cysts that I had previously

measured. (Before sectioning, I drew the outlines of the cyst with a Zeiss drawing-apparatus, and filled in also the nuclear outlines, when I could see them distinctly. Frequently I measured the nuclei at this stage, to act as a check on later measurements in section). The nuclei, when sectioned, I measured under oil-immersion. As in most cases the primary karyokinesis had been finished and the nuclei had begun to become heteropolar, I took measurements in two directions. This brought out an average, in the warmth, of $16{\cdot}6\ \mu \times 13{\cdot}8\ \mu$, and, in the cold, of $15{\cdot}2\ \mu \times 14{\cdot}8\ \mu$. That



TEXT-FIGS. 1 *a* AND 1 *b*.

Four primary cysts from culture B 9, arranged so as to show the comparative dimensions of cyst and nucleus in the warmth, 25° C. (darker line), and the cold, 10° C. (thinner line). Drawn with Zeiss drawing apparatus, oc. 3, obj. 7 (microscope tube of normal length), at the level of the work-table.

is to say, the mean diameter in the warmth was $15{\cdot}2\ \mu$, and in the cold $14{\cdot}8\ \mu$. Nuclei measured previous to the primary karyokinesis gave, in the warmth, an average diameter of $20{\cdot}2\ \mu$, and in the cold of $19{\cdot}5\ \mu$. In fact, the difference in size between nuclei from warm and cold cultures was scarcely appreciable; those from the cold culture were very slightly the smaller. In comparison with the considerable size-differences between the cysts themselves, this agreement in the dimensions of their respective nuclei is rather striking.

Though fairly exact correspondence in nuclear dimensions

seems to obtain, irrespective of temperature, yet I incline to think that, in the cold culture, the chromatin content of the individual nuclei is greater than in the warmth. Mr. Smith, in his article already quoted, also finds that "in cysts built in the warmth the amount of chromatin is absolutely, as well as relatively, less than in the other cases." He disregards, however, the comparative sizes of the nuclei in his cultures, considering that "such change of size" (of the nucleus) "could be brought about by an alteration in the conditions of tension in the cell, without any deeper changes in the physiological relations."

That the matter can be so lightly dismissed I do not believe, from what I have seen of the constancy in the relative nuclear-cytoplasmic proportions in my warm and cold cultures. An explanation is suggested by Professor Hertwig's Kern-plasma Relation. I must here recapitulate somewhat. According to Professor Hertwig, an encysting Actinosphærium loses about 95 per cent. of its nuclei in the earliest stages of encystation. Probably only 5 per cent. of the original nuclei will survive till the end of the mother-cyst stage, and pass on to further development. The manner of this nuclear elimination is not yet clear. Hertwig considers it unlikely that the reduction is usually the result of fusion; and he also holds it for improbable that, under normal conditions, nuclei are thrust out bodily. To this latter point I shall return later. What one actually sees is a gradual shrinkage of the nucleus; its membrane hangs round it like a loose sac, and it dwindles from about 14 μ to 6 μ or less. At the same time the central chromatin-rosette becomes less and less distinguishable, and the nucleus stains almost uniformly. In this "dead" condition it may remain for some time, but, under normal conditions, it disappears entirely before the primary cysts are formed.

Professor Hertwig's theory of "Kern-plasma Relation" gives a possible reason for this nuclear reduction. He supposes a sort of mutual antagonism to exist between nucleus and cytoplasm, such that, during high function in the cell,

the nucleus grows at the expense of the cytoplasm, while, during periods of rest, the cytoplasm exerts a "reducing" influence on the nuclear mass, bringing it back to its normal proportions. Hertwig sees in the maintenance of a certain definite proportional relation between nucleus and cytoplasm, fixed for each kind of cell, an absolute essential for the continuance of healthy cell-life. Anything that disturbs the equilibrium unduly, such as long continued feeding (when the nuclear element becomes abnormally enlarged), or starvation (when shrinkage of the cytoplasm is the disturbing factor), produces an injurious state of things that will result in death to the cell, if it be not corrected by nuclear reduction, and a consequent return to normal proportions. Consider the conditions used artificially to produce encystation—abundant nourishment, followed by complete starvation. Nothing could be more favourable to a great nuclear preponderance, and for this state of "depression" the organism seeks a remedy in encystation. As the nuclear growth had previously been excessive, so now the "reducing" power of the cytoplasm comes into play to an equally abnormal degree. Ninety-five per cent. of the orginal nuclei are "absorbed," and still further reduction is effected by subsequent formation of polar bodies.

In the cold culture in my experiment an undue proportion of nuclei survived the eliminating process, and, further, they were markedly rich in chromatin. A lowered temperature may be considered to have an inhibiting effect on nuclear reduction. This is probably also the cause of the relative slowness of the early stages in the cold culture. In the cold, nuclear reduction is slow and incomplete.

But there is every reason to believe, from the experiments of Hertwig and his students, that the "Kern-plasma Relation" is lowered or raised by the altering of the temperature conditions to which the cell is subjected. What would be excess of nuclear mass at a high temperature might not be in the least unfavourable to further development at low temperature.

The strongly-marked chromatin "haloes," however, round most of the nuclei in the cold culture, both before and subsequent to the primary karyokinesis, struck me as a later attempt to reduce the great nuclear preponderance to some extent by extrusion of a fine chromatin dust.

In one cyst from the cold culture I detected two nuclei. These are not the result of the primary karyokinesis, but are both primary nuclei, of unequal size (22·5 μ and 18 μ respectively). They lie closely apposed near the centre of the cyst, and are both very rich in chromatin (fig. 2). I regard this as still further proof of the unmanageably large number of nuclei remaining over in a case where lowered temperature has caused incompleteness in nuclear elimination.

Here and there I noticed the inclusion within the cyst-group of intensely-staining, round bodies, usually about 6 μ in diameter (fig. 3). At first sight these bear resemblance to polar bodies, but their occurrence at this early stage, the hint of a central chromatin rosette and of a shrunk nuclear membrane, decided me to class them as out-thrust dead nuclei from the mother-cyst stage. It is quite conceivable that, with the nuclear-absorbing power so much lowered by the cold, "dead" nuclei may easily escape complete dissolution in the mother-cyst, and may linger on through subsequent stages.

To sum up:

I. At a low temperature, Actinosphæria form small and numerous cysts, with nuclei scarcely below normal size, but markedly rich in chromatin.

At a high temperature, the cysts formed are large and few in number, with nuclei scarcely larger than those of the cold cultures, but poor in chromatin.

II. Lowered temperature paralyses the cell-functions to some extent. Nuclear elimination is slow and incomplete, as indicated by—

(1) The large number of nuclei retained from the mother-cyst reduction to act as centres for primary cysts.

(2) The superabundance of chromatin in these nuclei, as suggested by "haloes."
(3) The occurrence of two nuclei in one primary cyst.
(4) The occurrence of occasional "dead" nuclei within the groups of primary cysts.

Hitherto I have described the effect of cold on a culture of Actinosphærium that encysted quite normally. The majority of my cultures, however, encysted during the oncome of a "depression"-wave, and showed many abnormalities of structure. A few points noticed seem to be worthy of brief description. Full account of the effect of temperature on cultures of degenerate Actinosphærium will be given by my fellow-student, Miss Boissevain.

Where there was a tendency to abnormality it made itself noticeable in the cold culture first, as a rule—probably the next set of cultures would show abnormalities in the warmth. Finally, a stage would be reached when both sides of the culture would entirely refuse to encyst, or would encyst so abnormally and feebly that further development was impossible, and the cysts disintegrated in that stage.

Examination of non-encysted members of such cultures gives a clue to this condition. The organisms are undergoing a period of "depression." Prolonged function has led to an overwhelming nuclear preponderance, and, though by out-thrust of chromatin and nuclear fusion, attempts are being made to bring the relation back to the normal, yet the nuclear mass is so unmanageably great that the "reducing"-power of the cytoplasm is insufficient to cope with it, and, in extreme cases, the limits within which satisfactory encystation is possible, are never reached. Further, if it be granted that abnormal nuclear dimensions are injurious to cell-life, then the very presence of this pathological condition in the cell must render the cytoplasm still less equal to meet the heavy demand put on it.

Such individuals as succeed in establishing a certain amount of nuclear reduction may encyst; the rest of the culture will dwindle and die. The cysts formed show certain outward signs of abnormality, such as great unevenness in size within one cyst-group, irregularity of form, excessive development of silica.

Treated with reagents, and sectioned in the usual way, they are generally found to show corresponding abnormalities in the minute structure. Such abnormalities are exactly those observed by me in the cold "normal" culture B 9, but on a very much exaggerated scale. In both cases they may be looked upon as the result of a lowered vitality. In the normal culture this was induced by lowered temperature. In the other cultures it was the result of physiological degeneration.

Extreme examples of the condition may be induced by the co-operation of both factors, as is, indeed, shown by such of my degenerate Actinosphæria as encysted in the cold.

My culture series was too incomplete for me to make satisfactory comparisons from successive warm and cold cultures during the depression. I shall content myself with arranging the described abnormalities in the same order as that given at the end of my remarks on the normal cultures, adding a brief remark on the fate of the culture and its appearance in the other temperature. Such a scheme is, of course, rather artificial, but makes comparison easier, and brings my results together more compactly.

In an encystation culture of depressed Actinosphæria nuclear elimination tends to be incomplete.

(1) Too many Nuclei are retained to form Centres for Primary Cysts.—This means that the cysts formed tend to be smaller than the normal. I found, for instance, that $65\,\mu \times 55\,\mu$ was a very common measurement in the cold. There were, however, wide deviations from this, and, in the warmth especially, I noticed considerable irregularity of size. In such cysts the nuclei were of normal size or rather larger. The disproportion between the small cysts and their nuclei

was generally much greater than at first appeared, for the cytoplasm was often reduced to a minimum by extreme vacuolisation and by the great thickness of the silica coat.

The nuclear mass retained may be so much in excess of the available cytoplasm that development can proceed only up to a certain point.

(*a*) Disintegration may occur in the primary cyst. Fig. 4 shows one of a group of two primary cysts from the cold culture of B 8. The cyst pictured was of large size, 129 $\mu \times 79\,\mu$, but the silica was more than 10 μ thick, and the cytoplasm much vacuolated. So that the dimensions of the nuclei (the primary karyokinesis had already taken place), 19·5 $\mu \times 19{\cdot}8\,\mu$ in one case, and 18 $\mu \times 22\,\mu$ in the other, are excessive. The chromatin has been thrust out on all sides into the cytoplasm, and lies there in irregular strings, blotches and specks. The largest chromidia do not radiate from the nuclei, but tend to lie closely apposed to their surfaces in a tangential direction. The nuclei are almost devoid of chromatin, and show signs of shrinking. Probably further development was impossible.

Of the other cysts formed in this culture, one group made primary cysts with very large nuclei, and two more formed small vacuolated secondary cysts. The warm culture died off in the mother-cyst stage.

(*b*) Fig. 5 represents a cyst from the cold side of culture B 11. Here are nine nuclei (only six appear in the section figured) of very uneven size,—one is 32 $\mu \times 23\,\mu$, others measure 26 $\mu \times 15\,\mu$, 20 $\mu \times 17\,\mu$, etc., and two are under the normal size for this stage, 16 μ and 15 μ respectively. They are all rich in chromatin, and show vacuolated nucleoli. In contrast to the available mass, the cytoplasm is so scanty as to be almost absent round some of the nuclei. Silica has been excessively developed, and in such a way as to indicate that an attempt has been made to map out primary cysts, of which these nuclei are the centres. The power to separate these definitely from one another has been lost, and further development is scarcely possible. Several of the nuclei are surrounded by chromatin haloes, in which chromidia radiate

outwards, and in one case, that of the largest nucleus, the nuclear membrane seemed to have disappeared at one point, and the whole nuclear content is streaming out.

(*c*) No other member of this culture, B 11 (cold), got beyond the mother-cyst stage. In fig. 6 is shown one of these cysts. This shows elimination to have been so imperfect that a quite excessive number of nuclei remain; in the cyst figured there were 278. These have all shrunk and died, but remain as darkly-staining spots (6·5 μ to 9 μ in diameter), not near the borders of the cyst, but aggregated in little strings and groups. Each group lies in an "island" of normally-staining cytoplasm surrounded by non-staining brownish material, in which are shrinkage rents. This is an instance of "pycnosis," when the nuclei die off without being further disposed of, until the whole cyst disintegrates.

(The corresponding warm culture of B 11 showed such abnormalities as great fluctuation in size of cysts, and frequent occurrence of non-absorbed nuclei.)

(2) Occurrence of more than one Nucleus in a Cyst.—In Culture A 2 I noticed a few abnormalities in the warm and room temperature cultures, while the cold culture encysted only very feebly. In one cyst from the warm culture I counted as many as six nuclei (fig. 7). The cyst was the only one formed by the individual, and measures 120 $\mu \times 111$ μ, much of the bulk being due to the silica, which is about 18 μ thick. The nuclei are arranged in a group round a central point. They are of much the same size, 16 $\mu \times 14$ μ, and of markedly elongated form. On one side, generally that towards the exterior, they have shrunk away a little from the cytoplasm. At first, I took them to be heteropolar, but subsequent use of Delafield's hæmatoxylin failed to bring out any such arrangement of the chromatin. The nuclei are, in fact, singularly devoid of chromatin, and the nuclear reticulum is very faint, and free from chromatin aggregations of any size. In the cytoplasm lie also fourteen smaller darkly-staining bodies, measuring about 4 μ in diameter, and each surrounded by a vacuole. These remained unaffected by the hæmatoxylin

stain. In appearance they bear a very strong resemblance to polar bodies, but their number, fourteen, does not fit in with the number of the nuclei, which is six. Possibly, however, some of them may be the last remnants of "dead" nuclei. The Delafield's hæmatoxylin brought out clearly the broad zone of "Dotter-plättchen," just within the silica coat.

I was unable to decide as to the stage that this cyst has reached. I incline to think it a mother-cyst in which the nuclei have swelled out to the size of primary-cyst nuclei; but the power to form separate cysts round these has been lost.

(3) The Retention within the Cyst Group of Dead Nuclei from the Mother-cyst.—This occurs freely in most of the cases already described. In both warm and cold cultures the cyst-groups are very commonly accompanied by such nuclei in various stages of "shrinkage."

Fig. 8. Here is a group of seven primary cysts from B 2 (cold). The nuclei are in process of the primary karyokinesis. Twelve out-thrust nuclei remain round the group, and show still quite clearly their original structure.

Fig. 9 is a section from a group of twenty-four secondary cysts and conjugation-cysts from the room-temperature culture of A 2. Most of the cysts are in process of polar-body formation, but are not very clearly marked off from one another. Within the common cyst envelope are as many as forty-six nuclei of still quite appreciable dimensions.

I failed to observe out-thrust nuclei surviving till later stages than this.

I am inclined to think that nuclei are thrust out bodily from the early stages in the mother-cyst as soon as, from either of the causes suggested (i. e. lowered temperature and degeneration), nuclear absorption by the cytoplasm has been weakened.

In unencysted Actinosphæria Hertwig observes that, from such as have suffered hyperplasia or hypertrophy of the nucleus, parts of the organism, containing numerous nuclei, are thrust out bodily ('Physiolog. Deg. bei Actinosph.')

He speaks of nuclear out-thrust as occurring only "very abnormally" in the reduction in the mother-cyst. From what I have seen in my cultures I believe that, though certainly not quite normal, such out-thrust methods tend to come into play very quickly as partial substitute for a weakened power of nuclear elimination by the usual method. In all my cultures, except B 9 warm, I met with out-thrust unabsorbed nuclei till a certain stage in the degeneration was reached, when even this more drastic measure seemed unavailable, and the nuclei died off in the mother-cyst, remaining there unabsorbed until the whole gradually disintegrated.

I wish to express my warm thanks to Professor Hertwig and his assistants for their unfailing readiness with advice and practical help during my work in Munich; and also to Miss Boissevain for her many valuable suggestions and personal assistance.

Marischal College,
Aberdeen.
December, 1907.

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EXPLANATION OF PLATE 24,

Illustrating Miss Doris L. Mackinnon's paper on "A few Observations on the Encystation of Actinosphærium eichhorni under different conditions of Temperature."

(For Fig. 1 see text, p. 412.)

Fig. 2.—Occurrence of two nuclei in one primary cyst previous to primary karyokinesis. (B 9, cold.)

Fig. 3.—To illustrate occasional retention of dead nuclei in the common cyst-envelope. (B 9, cold.)

Fig. 4.—Chromidia in primary cyst after primary karyokinesis. (B 8, cold.)

Fig. 5.—Unsuccessful attempt to form primary cysts. (B 11, cold.)

Fig. 6.—Mother-cyst dying off, with 278 "dead" nuclei. (B 11, cold.)

Fig. 7.—One cyst containing six nuclei and 14 "dead" nuclei (?). (A 2, warm.)

Fig. 8.—Group of primary cysts, in course of primary karyokinesis, showing ejected nuclei. (B 2, cold.)

Fig. 9.—Group of conjugation-cysts and secondary cysts, in course of polar body formation, showing ejected nuclei. (A 2, room temperature.)

All, except Fig. 8, are drawn from sections 10 μ thick.

Fig. 8 is from a preparation still in toto.

All were fixed with picro-acetic acid and stained with borax carmine. 6 and 7 were afterwards stained with Delafield's hæmatoxylin.

All figs. are enlarged 193 diameters.

On Archerina, Golenkinia and Botryococcus.

By

Sir Ray Lankester, K.C.B., F.R.S.

With Plate 25.

A. Archerina Identical with Golenkinia and Richteriella.

In the year 1885 I described in this Journal—vol. xxv, new ser., p. 61—and figured in a coloured plate, a minute chlorophyllogenous organism, for which I formed the genus "Archerina." I named the species "A. boltoni." Nine years later[1] Professor Chodat, of Geneva, described the same organism under the name "Golenkinia radiata," in Morot's 'Journal de Botanique,' Tome viii, 1894, September 16th. Professor Chodat obtained his specimens from a small duck-pond in the public park at Geneva. My specimens were sent to me in a bottle-full of living material by Mr. Thomas Bolton, of Birmingham. I have now reason to believe that the "gathering" was obtained by Mr. Bolton from the duck-pond of the gardens of the Royal Botanical Society in Regent's Park, whence I have since obtained Archerina.

No one who compares my figures of the actinophrys-like form of Archerina (figs. 2, 4, 5, 6, 7, 13, 16, and 17 of Pl. 7, vol. xxv, 'Quart. Journ. Micr. Sci.,' New Series) with Chodat's figures and description can doubt that the organism figured by me is the same as that represented nine years later

[1] I am indebted for my knowledge of Professor Chodat's memoirs to a most valuable and compact little volume on the 'British Freshwater Algæ,' by Mr. G. S. West, published in the Cambridge Biological Series, 1894.

by Chodat (compare especially fig. 2 of his Pl. III). Not only are the radiating processes and the form of the chlorophyll bodies identical in the two sets of figures, but Chodat also figures and describes the empty, discarded spherical cases with radiating processes, which I described under the name of "ghosts," and figured in fig. 18 of my Pl. 7.

In examining this organism in 1884 I was particularly struck by the frequent association with it of colourless, naked amœboid protoplasm, which I was led—I now think erroneously—to consider as an essential part of the organism itself. I now believe that this amœboid protoplasm belonged to a Vampyrella-like organism which associated itself with the Archerina, and frequently invested it so closely as to lead to the supposition that it was part of the Archerina itself. I have since come across several cases of this close investment of a minute algoid organism by the naked protoplasm of an amœba-like or Vampyrella-like companion. A case which I may mention is that of the hollow botryoidal fronds of the interesting Clathrocystis æruginosa of Henfrey, which I have had very ample opportunity of studying.

I have no doubt that it is due to the fact that I was led, by the association of extraneous amœboid protoplasm with many specimens of Archerina, to refer this organism to the Protozoa, that my description of it has escaped the notice of Professor Chodat and other botanists. Nevertheless, I think that the genus Golenkinia and the species G. radiata must give way to the genus Archerina and the species A. boltoni of nine years' earlier publication.

The name Phythelios given by Frenzel in 1891 to what is probably the same organism is also later than Archerina.

Whether there is anything like a constant or very frequent association of Archerina with a minute amœboid commensal remains an open question.

Subsequently to Chodat's description of Archerina as Golenkinia another botanist, Lemmermann, described (in 'Hedwigia,' Bd. xxxvii, 1898, p. 303) under the name "Richteriella botryoides," some of the phases of divi-

sion of Archerina, which were described and figured by me in my paper of 1885. These are the symmetrically-grouped division-products of Archerina drawn in figs. 21 and 22 of my paper (Pl. 7, 'Quart. Journ. Micr. Sci.,' vol. xxv). I would further draw attention to the oblong form of the chlorophyll bodies shown in my fig. 21, as agreeing with some of Lemmerman's figures of another supposed new genus of his.

It is, of course, possible to maintain that these oblong bodies are specially distinct from the more usual spherical forms, but I do not think that there is any sufficient ground for separating, generically or specifically, the much divided groups of small-sized spheres from the larger single spheres of Archerina boltoni with which they were associated and with which they are connected by intermediate phases of division, as well as by the characteristic radiating processes of the cell-envelope.

In my judgment Golenkinia and Richteriella are synonyms of Archerina, and I think this will be admitted by botanists who compare my plate of 1885 with the much later drawings of Chodat and of Lemmerman. I am at the same time of the opinion that the reference of Archerina to the Protozoa by me was an error, and that the organism is to be regarded as one of the simpler Protophyta.

B. Botryococcus.

Curiously enough it is to a paper also published by Prof. Chodat in Morot's Journal at a later date, 1896, that I am indebted for the identification of a very beautiful minute fresh-water organism which I studied and drew about the same time as that in which Archerina came before me. I used to speak of this as the "Cayenne pepper growth," since it appeared as little grains resembling in colour and size those of that condiment, floating in closely packed aggregates on the surface of the English Lakes (Grasmere and Derwentwater). I received it first in 1884 from Mr. Bolton, of Birmingham, and some sixteen years later from

my friend Prof. Hickson, F.R.S., of the University of Manchester. I was at first unable to identify it, but suspected it to be the Botryococcus of Kützing (1849), a suspicion which I was unable to confirm owing to the fact that no good figures of it were published. In 1896, however, Prof. Chodat published a coloured plate (Pl. III, p. 333) in Morot's 'Journal de Botanique' (which only came to my knowledge last year) accompanying a full account of the Botryococcus Braunii of Kützing, which he found abundantly and at various seasons of the year on certain parts of the surface of the Lake of Geneva. Prof. Chodat describes, and his figures illustrate, a purely green form of this organism, a phase which I have not seen. But he mentions that frequently the Botryococcus develops a brick-red coloured oil, which may be more or less abundant, and give a completely red appearance to the floating colonies. He points out that the red oily matter enables the organism to float, and expresses some doubt and interest as to the exact mode of formation of this red-coloured oil.

Whilst referring the reader to Prof. Chodat's memoir for many interesting observations, I will now briefly describe my own observations and the drawings made by me nearly twenty-five years ago, which I have never published, but now reproduce in Pl. 25 accompanying this paper.

General form and colour of the fronds.—The little "grains" of Botryococcus which float practically on the surface of the water in which it occurs are irregular, incomplete, hollow, spherical, or kidney-like bodies, connected one to another by growth and origin, and separating by rupture from one another after a certain size and shape has been attained. A group of these growths magnified about one hundred diameters is shown in outline in Pl. 25, fig. 4. In fig. 1 of Pl. 25 a smaller frond is shown more highly magnified. This drawing also serves to show the very striking coloration of the first specimens which came under my notice, viz. a golden-red mass with a translucent green-coloured cortex. The colouring is so strong as to recall the

beautiful tints of uranium-glass and to suggest the adjective "chryso-chlorous" to describe this green and gold phase of vegetation. A more highly magnified view of a portion of the edge of the frond as seen when under the pressure of a cover-glass (Pl. 25, fig. 3) shows that the two colours are due (*a*) to the green colour of the granules, and to some extent of the cell-substance of the cell-units which build up the organism and form its superficial layer; and (*b*) to the orange-yellow or often brick-red colour of the jelly which is formed by the cell-units and holds them together.

As shown in fig. 2 and fig. 3 of Pl. 25 droplets of a more or less oily nature are pressed out of the jelly when a cover glass is laid over it.

In many specimens I found the cells pale green with green granules whilst the jelly was brick-red. In other cases the cells were as just stated, but the jelly was yellow. I did not find any instances in which the jelly was green in colour, though Prof. Chodat has figured the fronds in that condition.

In some examples I found that, whilst the general substance of the cell had a sage-green tint, the granules were orange-yellow. These are shown in Pl. 25, figs. 6 to 10. In some specimens, which were of very strong brick-red colour, the cells contained a very large quantity of orange-red granules (Pl. 25, figs. 11, 12, and 13).

I do not gather from Prof. Chodat's account of Botryococcus that it has ever been shown that the green colouring matter is chlorophyll. My own impression, based chiefly on the sage-green tint, was that it was not. But the nature of the green pigment seems not to have been definitely determined. I mention this because in an organism which has very much smaller cell-units, but forms similar hollow botryoidal colonies, namely, Clathrocystis æruginosa, Henf., the green colour, though the alga is of an intense apple-green when collected in mass (as a sort of cream) is certainly not due to chlorophyll, but to a peculiar body insoluble in alcohol and changed by ether into a brown pigment. The same

peculiar green pigment occurs in Aphanizomenon flos-aquæ, with which I believe Clathrocystis to have a special relationship. This pigment is remarkable for changing, when dried with exposure to light and air, from an apple-green to a blue verdigris-green tint.

The jelly.—The cell-units of Botryococcus form a closely-set superficial layer one cell deep. They secrete a jelly-like material which forms a denser capsule to each cell (Pl. 25, figs. 16 and 18), and is of a softer more watery consistence below that layer and between the adjacent capsules. Under slight pressure the capsules burst, and the cell itself is shot out of its position in the jelly into the surrounding water. The capsules burst by dehiscence of a concave-convex lid (fig. 18).

In the parts where one sub-spherical or kidney-shaped mass is adherent to similar neighbouring masses the jelly is often broken up into fibrillated strands (Pl. 25, fig. 5), the formation of which seems to be connected with the division of one original colony into separating sub-colonies.

The colour of the jelly in all my specimens was either golden-yellow or a deeper brick-red. I gather from Prof. Chodat's description that it may present itself as entirely colourless or with a greenish tint.

I made no chemical tests of the nature of this jelly, but some are recorded by Chodat.

The cell-units.—These as shown in the figures in Pl. 25 are oblong and somewhat pyriform. They consist of a continuous dense substance, which rarely exhibits vacuoles (fig. 17). From one to forty sharply marked granules are embedded in the dense substance and these granules are in some specimens green (fig. 3), in others they are yellow (figs. 9, 10), in others brick-red (figs. 11, 12, 13).

The cells of Botryococcus when extruded from their capsules and the adjacent jelly exhibit no movement. They are devoid of flagellum or cilia.

In the living cells it is not possible to observe any structure in the cell representing a nucleus, but I found in

specimens stained with hæmatoxylin en masse for several hours that a central substance exists which shows a deep purple stain (see Pl. 25, fig. 14). I did not detect any characteristic nuclear structure in this in specimens clarified and mounted in the usual way, but I am not in a position to say that such might not have been present though it escaped my observation.

The cells appear to divide by binary fission along the long axis (figs. 15, 17). After fission the resulting cell-units separate and recede to a distance from one another equal to the short diameter of the cell, the interspace being filled by jelly (fig. 18).

It is a long time since I had the opportunity of observing this organism, and I should urge those who may meet with it in the Lake district or elsewhere to direct their attention to the following points:

(*a*) The nature of the green colouring matter.

(*b*) The relation of the variable amount of yellow and red oily pigment to the season.

(*c*) The mode of passage of the colouring matter into the jelly.

(*d*) The existence of specimens showing colourless and of others showing green-coloured jelly.

(*e*) The nuclear structure.

(*f*) The possible occurrence of other modes of reproduction than the longitudinal fission leading to increase in the size of colonies.

It does not appear that more than one European species of Botryococcus can be distinguished. The genus Ineffigiata of Mr. West is, I am informed by him, probably based upon a local growth variety of Botryococcus Braunii.

May 31*st*, 1908.

EXPLANATION OF PLATE 25,

Illustrating Sir Ray Lankester's Memoir "On Archerina, Golenkinia, and Botryococcus."

Fig. 1.—A floating colony of Botryococcus Braunii, Kützing, of the chrysochlorous variety. Magnified about 100 diameters.

Fig. 2.—Portion of a colony under cover-glass pressure, superficial focus. More highly magnified.

Fig. 3.—A portion of a reddish colony. Still more highly magnified.

Fig. 4.—Outline of a series of contiguous hollow reniform colonies, as found floating on lake-water. Magnified about 50 diameters.

Fig. 5.—Portion of neighbouring reniform masses more highly magnified to show the fibrillated strands of jelly connecting them to one another.

Fig. 6.—Cell in the gelatinous investment, with yellow granules.

Fig. 7.—Cell with displaced capsule-lid.

Figs. 8, 9, 10.—Similar cells to that shown in Fig. 6.

Figs. 11, 12, 13.—Large cell units with abundant orange-coloured granules.

Fig. 14.—Five cells treated with alcohol, followed by hæmatoxylin and alum-staining-fluid, and mounted in balsam. A dark stained central body is seen.

Fig. 15.—Cells in binary longitudinal fission. Observe the vacuoles.

Fig. 16.—Cell with strongly marked capsule.

Fig. 17.—Cells in binary longitudinal fission, as in Fig. 15.

Fig. 18.—Two adjacent cells to show the shell-like capsule of each, the interposed jelly, and the capsular lid of each cell reflected owing to pressure, and about to allow the cell to be ejected as a naked isolated unit.

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The Yellow-Brown Cells of Convoluta paradoxa.

By

Frederick Keeble, Sc.D.,

Dean of the Faculty of Science and Professor of Botany in University College, Reading.

With Plates 26—28, 3 Text-figures, and 2 Tables.

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Section I.—Introduction.

Convoluta paradoxa (= C. convoluta, Auct.) is a small brown acœlous Turbellarian which lives among the sea-weeds of the shore. The characteristic colour of the animal

is due partly to orange-red glands occurring in the superficial tissues, but chiefly to numerous yellow-brown cells, which are distributed fairly regularly in the sub-epidermal and deeper tissues of the body.

Though excellent descriptions have been published by von Graff (1891) of the habits and structure of C. paradoxa, nothing appears to be known with respect to its yellow-brown cells. The origin, development, significance, and fate of these cells are alike obscure.

It is chiefly with these yellow-brown cells that the present paper deals.

The investigations on which the paper is based were begun by the writer in collaboration with Dr. Gamble.

Of the conclusions enumerated in the summary, those to which the letters "G and K" are appended are the result of our joint work. For the others the writer of the paper is alone responsible. The research has been conducted in the laboratory, Trégastel, Côtes du Nord, and at University College, Reading.

Section II.—The Bionomics of C. paradoxa.

(*a*) The Paradoxa Zone.—Convoluta paradoxa has its habitat among the finer brown and red seaweeds which occur at some little distance below the low-water mark of all but the larger spring-tides (Pl. 26, fig. 1).

The animal is flattened dorsi-ventrally; its anterior end is somewhat blunt, whilst posteriorly the body is prolonged into a slender tapering tail (Pl. 26, fig. 5; Pl. 28, fig. 10). The lateral margins of the body are flexed ventrally, and form, with the ventral surface, a groove whereby the animal is fitted saddle-wise over the weed on which it glides. It progresses by a gliding motion, and whenever it meets with some minor obstacle the sides of the saddle-like flexure give way and adjust themselves once again to fit the surface over which it is passing. If the animal encounters a more serious obstacle it fixes itself by its tail-end, partly by a mucilaginous secre-

tion and partly by bristle-like pegs, which stand out from the delicate cilia with which the body is clothed, and which are more numerous in the posterior region. Thus fixed, the anterior end is reared up caterpillar-wise, and the ventral surface fitted again to the substratum, or, quitting the substratum, the animal may swim freely in the water.

Though by no means gregarious like C. roscoffensis, C. paradoxa may be taken in fair quantity by following down the big tides and washing the finer weeds which it chiefly affects into a white porcelain dish, or by collecting the weeds, bringing them into the laboratory, taking them piece by piece and holding them so that the water drains down from them into a white dish. The animals follow the water draining from the weed, and so collect in the dish below. A particularly good catch may result in the collection of a hundred or more specimens. It is advisable to follow the falling tide, since many of the animals desert the weed as the tide falls off it, whilst those that remain cling so obstinately to the weed that even vigorous shaking fails to dislodge them. A white dish is better for the purpose than a transparent glass vessel, since C. paradoxa is much more easily pipetted off from the former than from the latter.

The sequence of sea-weeds on the rocks at Trégastel proceeding toward the sea is:—Pelvetia : Fucus, with a fine yellow-brown epiphytic algal flora attached to the fronds of Fucus on the seaward side; Ascophyllum : Himanthalia, the long strands of which, only exposed at fairly low tides, are also clothed toward their extremities with finer brown and dull red weeds; and, in the deep water, rarely and then but partially exposed, Pycnophycus, whose rounded thallus is covered with delicate red weeds, the chief of which are species of Ceramium and Rhodomela.

The limits of the Paradoxa zone are, on the landward side, the lower edge of the Fucus zone, and, on the seaward side, a little before the line which marks the permanently submerged part of the Pycnophycus formation. Within this zone the distribution of C. paradoxa varies with the phase

of the tides. At the onset of the spring tides the animals, chiefly immature specimens, may be found among the fine yellow weed attached to Fucus on its seaward edge. During the succeeding tides C. paradoxa moves seaward, and must be sought among the delicate weeds attached to Ascophyllum, whilst still later in the same series of spring tides it is only to be found among the Ceramium and Rhodomela and similar red weeds which cover the cord-like thallus of Pycnophycus. The same weeds permanently submerged a few yards further from the shore yield no animals. At both upper and lower limit of its zone of distribution C. paradoxa is represented only by immature, minute forms.

(*b*) Migrations—Tropisms within this Zone of Distribution.—There is, during the large spring tides, a tidal migration of Convoluta. The animals follow the falling tides seaward, and the rising tides landward. A study of the behaviour of the animals in the laboratory provides the probable explanation of this "ebb and flow" movement.

As has been mentioned, Convoluta tends to let itself be carried downward by the water draining off the weed. An explanation of the tidal, seaward movement based on this fact, sufficient as it seems at first sight, does not account for the zonal distribution of the animals, nor for all the facts of their "ebb and flow" movement. Thus for a little while after the water has left the weeds of the Paradoxa zone, animals may still be found in fair numbers among them. Later C. paradoxa deserts the weeds, and may be caught in the act of doing so by catching the drip from them. Closer examination of the question shows that a number of other factors co-operate in determining these tidal migrations. These factors are—(1) response to contact with weed or other solid body; (2) behaviour in stillness and darkness; (3) background reaction; (4) phototactic reaction.

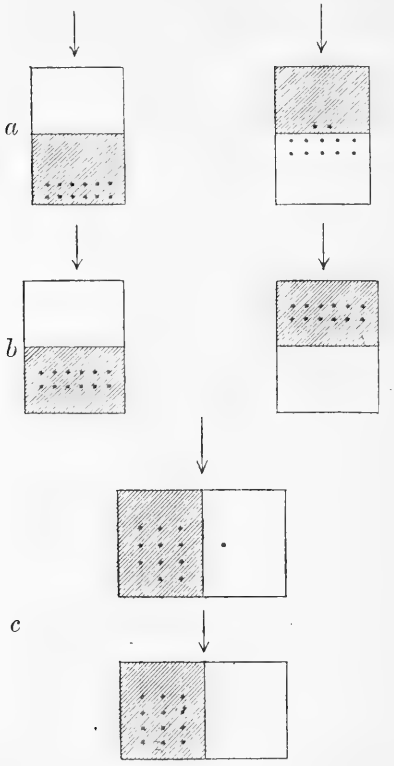
(1) and (2) Response to Contact with Weed, etc.—Convoluta exhibits the reaction of thigmotactism, that of clinging to a solid object, in a marked yet peculiar fashion.

The body, fitted to the weed in the manner already described, is dislodged from it with difficulty. Nevertheless there are conditions under which C. paradoxa relaxes its hold and becomes a swimming and no longer a gliding animal. Among these conditions the chief are change in light-intensity and change in background. Under yet other circumstances C. paradoxa relaxes its hold on its substratum, and becomes temporarily at least an animal of plankton habit, floating passively on the surface-film of water buoyed up by a mucilaginous secretion. Stillness of the water and darkness, particularly if seaweed is absent, bring about this behaviour. Animals placed in a dark vessel exhibit this phenomenon of "upness" with uniform regularity. It is not a pathological phenomenon, since it is manifested readily by fresh-caught animals, and since animals floating thus on the surface-film at once respond to stimulation, descending on exposure to light or, and yet more readily, on a slight disturbance of the surface of the water.

The "up" position is assumed whether the upper surface of the water is exposed to the air, or whether, contained in an inverted glass vessel, the upper surface of the water is against the glass. If weed is present in the vessel the animals for the most part do not leave it, though occasionally some assume the "up" position. Though more marked in darkness, this habit of floating on the surface-film is also exhibited by animals kept in the light, particularly if the light is feeble. We must conclude that, in the absence of any orientating stimulus whatever or of one of sufficient intensity, C. paradoxa leaves the "ground," rises to the surface-film, and floats there passively. Like all such movements, this at the present time is as inexplicable physiologically as ecologically it is obvious. Anticipating the evidence (p. 453) proving that C. paradoxa is dependent on light for its existence, we recognise that, borne out of its zone by currents or tides, it has, by virtue of this habit of "upness," a chance of regaining its home or of maintaining its existence in a new region; without it, condemned to hold

to the dim sea-bed beneath deep water, its light-requirements would fail to be satisfied, and it would perish.

"Upness," then, is a habit, and, like any other habit, it



TEXT-FIGURE 1.—Phototactism of C. paradoxa: the influence of background on phototactic response. The flat, porcelain troughs containing the animals are represented (in plan) by the oblongs. The bottom of each trough is half white and half black. In the diagram the white ground is indicated by the unshaded, the black ground by the shaded part of the oblong. The animals are represented by dots, and arrows show the direction of the light.

a. In bright light.

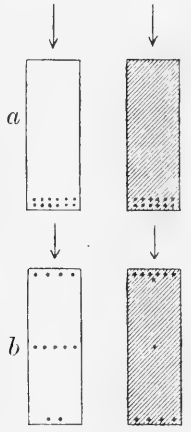
b. In weak light.

c. "Choice" of black ground in preference to white ground.

may manifest itself in opposition to weak stimuli, which, of themselves, tend to produce an opposing movement, or it

may be overridden by strong stimuli. Under normal conditions, whilst C. paradoxa keeps well within the region of a perpetual rain of powerful directive stimuli, "upness" will not manifest itself. Under abnormal conditions the habit will assert itself, and, on the average, to the benefit of the animal.

(3) Background Reaction.—C. paradoxa tends to stick to a black ground and to move freely over a white



TEXT-FIGURE 2.—Phototactism of C. paradoxa: the influence of light-intensity on phototactic response. *a*. Mode of response when the light-intensity is high. *b*. Mode of response when light-intensity is low. The glass troughs containing the animals are represented (in plan) by oblongs. The troughs standing on a black ground are represented by the shaded, those on a white ground by the clear oblongs. The animals are indicated by dots, and the arrows show the direction of the light.

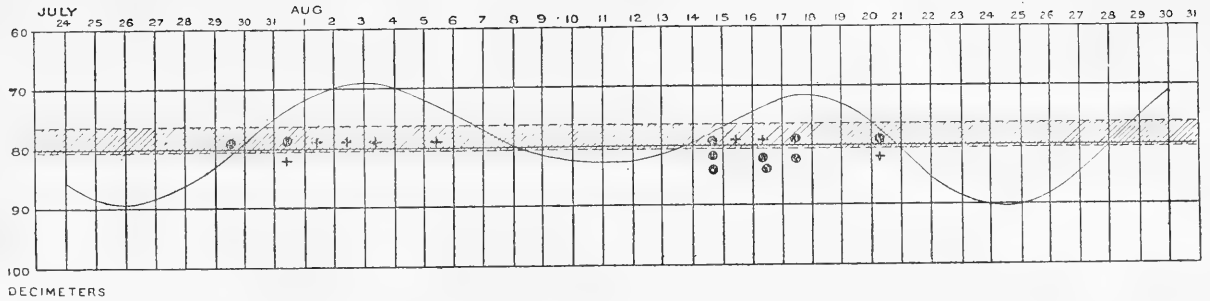
ground. It is probable that the sticking is due to light-perception by the orange-red "glands" of the body (Pl. 26, figs. 4 and 6), and particularly of the tail and to consequent reflex secretion of mucilage and activation of the stout bristle-like structures which, as described, project from the surface of the body. It is significant that the tail-like extremity of the animal is at once richer in pigment-containing glands and in bristles than is the rest of the body, and that it is also the sticking-organ.

When offered the alternative of white or black ground C. paradoxa takes up its position rapidly and permanently on the latter (text-fig. 1 *c*).

(4) Phototactic response.—C. paradoxa is negatively phototactic in light of fairly high intensity; for example, in the north light of the laboratory. This reaction is demonstrated readily by the usual methods (text-fig. 2). In light of low intensity the sign of the reaction changes, and the animals become either feebly positively phototactic or aphototactic (text-fig. 2 *b*).

The intensity of light is not, however, the only factor which modifies the mode of response of the animal to the directive influence of light. As is the case in so many other littoral animals (Gamble and Keeble [1903], Keeble and Gamble [1904]) back-ground may also modify phototactism. This is readily demonstrated by putting equal numbers of animals in each of two troughs the bottoms of which are half black and half white. When such vessels are placed in a good light, one with the white half toward the source of light, the other orientated in the opposite way, the animals exhibit their negative phototactism more rapidly if the movement involves a passage from white to black than if it requires a passage from black to white ground (text-fig. 1 *a*). Directive reaction masters back-ground reaction here though the latter produces an effect. When, however, the same experiment is carried out in dim light the tables are turned, and back-ground reaction dominates directive reaction (text-fig. 1 *b*). Thus in dim light the animals accumulate on the black ground no matter whether the black half of the dish is toward or away from the source of light.

Now to apply these facts to account for the zonal distribution of C. paradoxa, and to explain the "ebb and flow" tidal movements of the animals within the zone. Experiments described in Section III prove that C. paradoxa cannot flourish in darkness, and that it requires light of a certain intensity in order to carry on its nutritive processes. Now the zone in which C. paradoxa lives is characterised, speci-



TEXT-FIGURE 3.—Periodicity of egg-laying and hatching of C. paradoxa. The shaded band shows the position of the Paradoxa zone with respect to low water-marks of spring and neap tides.

The undulating line, joining up the low water-marks of successive day-tides, is obtained by marking off, along the verticals indicating successive days, from a zero line above, the amount of vertical descent (in decimeters) of each day's tide.

On those days when the undulating line falls below the shaded band the Paradoxa zone is uncovered during low water; on those days when the low water-line lies above the shaded band the zone is continuously submerged.

The dots represent egg-capsules, the crosses signify larvæ hatched; the positions of dots and crosses give the dates on which the capsules were laid and larvæ emerged.

ally during the large spring tides, by a periodic change in the amount of light to which it is exposed. As the tide is falling off the upper limit of the Paradoxa zone the animals are exposed to an increasing intensity of light and to an increasingly lighter background. The latter factor causes them to release their hold on the weed, and so to let themselves go with the water draining from the now exposed weed. Other animals turn their negative phototactism to a like account and, failing escape in the way just indicated, creep into the thickness of masses of weed and attach themselves firmly in obscure situations on the dark ground which the weeds provide. Those which follow down the current will be arrested by the weeds of a lower level; thigmotropism and darker background will tend to check their course and high light-intensity to drive them on. The position assumed will be the physiological resultant of these opposing forces. The return movement with the incoming tide will result in the animals once again taking up the most favoured light-position. This most favoured light-region will shift seaward with the increasing spring tides, and return again landward as these tides fall off.

(*c*) Periodicity of Egg-laying.—The periodically changing conditions under which C. paradoxa lives induce rhythm not only in the migrations of the animals within the Paradoxa zone, but also in the period of egg-laying.

Animals were collected daily, whenever the tides allowed the Paradoxa zone to be approached from the shore, during the months of July and August, 1907. The results showed many minute, mid-sized, and large animals, but scarcely any mature females. That the eggs are laid within the zone there is no doubt, since, occasionally, capsules are found attached to the weed of the zone.

The scarcity of mature females is due to the fact that maturity is reached and egg-laying effected only during certain tidal periods.

The results supporting this conclusion are displayed in text-fig. 3, which records the dates on which egg-capsules

were observed, and also the dates of hatching of the larvæ in the laboratory. The undulating line in the diagram represents the magnitude (in decimetres) of successive daily tides from July 24th to August 30th, and is based on the tide-almanac for Trégastel. The shaded band represents approximately the extent of the Paradoxa zone. The position of the zone is such that it is fully exposed by a fall of 80 decimetres, whereas a fall of 76 decimetres leaves it submerged.

Where the undulating line falls above the shaded band in the diagram the zone is submerged, viz. July 30th to August 8th, and August 14th to 21st; where the tide-line falls below the shaded band the zone is uncovered twice daily during low water. Each black dot records the date on which an egg-capsule was found either in the open attached to the weed or, and this was the case in the large majority of the records, was laid in the laboratory by C. paradoxa kept under as normal conditions as possible. Each cross marks the date on which a batch of larvæ hatched out in the laboratory.

The relation between time of egg-laying and tide is at once apparent, and may be stated thus :—Egg-laying occurs only at periods when the zone is submerged for six or seven successive days, i. e. during neap tides.

Hence it is that, during the spring tides, only immature animals are to be met with, and that supplies of egg-capsules can only be obtained by collecting the submerged weed during neap tides or by keeping the animals in the laboratory.

It must remain for the present an open question whether this phenomenon of periodic egg-laying is to be interpreted as a physiological consequence of the more active nutrition which obtains during exposure to the higher light-intensity of the preceding spring tides or whether it must take its place in the innumerable crowd of biological phenomena of adaptive significance (compare Keeble and Gamble, 1907). It is evident, of course, that during the slack tides the eggs, being permanently submerged, are protected from the risk of dessication, and to some extent from shock to which they would

be exposed if they were deposited during the springs when the zone is laid bare at each tide and receives the full force of the incoming waves.

Another noteworthy point is the rapidity with which the larvæ hatch. Within 24—48 hours after the capsules have been laid, the young emerge. Thus the majority of C. paradoxa are, during the first few days of their existence, continuously covered by water, and during this time also their habit of clinging to the weed is but ill-developed. Soon after the larvæ emerge the yellow-brown cells appear and multiply rapidly in the body and the animals develop or acquire those tropisms whereby they maintain themselves, despite the recurring changes of tide and consequently of illumination, within the narrow limits of the Paradoxa zone.

(*d*) The Eggs and Larvæ.—The eggs of C. paradoxa are laid in clutches contained in a common transparent capsule, each egg being likewise enveloped by a thin membrane (Pl. 26, figs. 1, 2, 3). The common capsule is less mucilaginous than that of C. roscoffensis, but sticks very firmly to any object on which it is deposited. The number of eggs in a capsule varies very considerably from as many as twenty-five to half or even less than half that number. By reason of the pigment of the eggs the capsules appear of an orange or pink-orange colour. The amount of pigment in the eggs varies very considerably. Its formation does not appear to be dependent on light, since eggs laid by animals kept for many days in darkness are as deeply pigmented as those produced by animals in normal situations. The pigment consists of small oval or dumb-bell shaped granules, each of which presents the appearance of a double-contoured band of orange pigment with a clear inner part. These pigment-bodies are probably the forerunners of the orange-pigmented glands of the adult animal. In addition to those granules which are distributed with fair uniformity, aggregations of finer granules of pigment may also occur in the egg. It was doubtless owing to the presence of the larger orange granules in the egg and larva that von Graff was led to the conclusion

that the yellow-brown cells of C. paradoxa are present in the egg.

Careful examination shows, however, that this view is erroneous, and that the orange granules of the egg have no relation whatever with the yellow-brown cells of the larva or adult. The pigment-bodies of the egg have none of the histological features of the yellow-brown cells of the larva or adult; the pigments are distinct in colour and react differently with reagents. Thus, whereas the orange granules of the egg and also the orange glands of the animal become colourless when treated with 90 per cent. alcohol, the yellow-brown cells of the animals become green. In other words, these cells contain two pigments, a yellow and a green, whilst the orange granules contain only one pigment. Further proof that the orange granules are in no way whatever related to the yellow-brown cells is given in Section III.

(*e*) Ingestion of solid food by C. paradoxa.—Immediately after hatching C. paradoxa like C. roscoffensis begins to ingest solid bodies (Pl. 26, fig. 4).

But whereas the latter species, soon after its infection by the green cells, ceases altogether from ingesting solid food, C. paradoxa continues throughout its life to be a voracious feeder. Its staples of diet are diatoms, many dozens of which may often be found in all stages of digestion lying in digestive vacuoles (Pl. 26, fig. 4). In addition to diatoms C. paradoxa takes up almost any small organisms it comes across. It also swallows and digests relatively enormous copepods; whilst later in the year its body may become so studded with the tetraspores of various algæ as to give to the animal a dark red colour. Failing suitable food-bodies C. paradoxa swallow greedily any inert, granular substance supplied to it. When supplied with congo red, masses of this pigment are ingested, and lie in colourless vacuoles in the solid gut. The congo red retains it colour in these vacuoles, indicating that, though ingestion does not require any chemical stimulus, the secretion of digestive juices into the vacuoles depends on such a stimulus. It is probable that the digestive secretion

acts in an acid medium, since, when under certain circumstances to be described presently the yellow-brown cells undergo digestion in vacuoles, the space between the wall of the vacuole and the cell undergoing digestion is occupied by a faintly pink fluid. The solid food is taken in through a mouth which is not recognisable when closed, but which is seen during the act of ingestion to be capable of an enormous gape (cf. Bronn's 'Tierreich,' iv, 68—71).

The solid gut which consists of parenchymatous tissue has no clearly defined outline; but its shape may be traced roughly by the positions which solid bodies fed to the animals take up in it. A convenient substance for this purpose is uric acid, the crystals of which are recognised readily, and which, unlike many inert substances, does not seem to provoke excretion. Not only may large quantities of this substance be ingested, but the crystals remain for a very long time in the digestive vacuoles. By such means it can be seen that a short broad gullet passing back from the mouth gives off two successive pairs of transverse processes, and then ends in a broad but gradually tapering mass of granular tissue, which reaches almost to the posterior end of the animal. The lateral processes are connected with prolongations which run one on either side of the body forward almost to the level of the statocyte and also a short distance backward. It is noteworthy that the two broad and prominent transverse bands of dark refractive material (concrement granules), Bronn (loc. cit.), which are so characteristic of C. paradoxa (Pl. 26, figs. 4 and 5), and which von Graff regards as being of the nature of nitrogenous excretory substance, lie over the two pairs of transverse processes which connect the gullet with the large laterally placed parts of the gut.

Mention may be made here of several attempts to infect larval C. paradoxa, whose bodies were free from yellow-brown cells, with the green, flagellated infecting organism of C. roscoffensis. The flagellated cells were ingested by C. paradoxa and transferred to large vacuoles, where they remained intact for several days with their eye spot and

pyrenoid sharply defined. After a further period, however, the green cells gradually disintegrated, and the attempt to effect what, if it had succeeded, would have been a synthesis of no small interest proved abortive.

Section III.—The Yellow-brown Cells.

(*a*) Description.—In every specimen of C. paradoxa taken from the sea yellow-brown cells are present (Pl. 26, figs. 5 and 6; Pl. 27, figs. 7, 8, and 9). These cells are as constantly characteristic of C. paradoxa as are the green cells of C. roscoffensis. Though not so numerous as to give, when examined microscopically, the appearance of a continuous tissue as is the case with the green cells of C. roscoffensis, the yellow-brown cells of C. paradoxa are, nevertheless, present in the adult body in great numbers. They occur in groups or rows situated for the most part just beneath the epidermis, though they also occur in the deeper tissues. The number of yellow-brown cells increases with the size of the animal. Very minute young specimens contain but few, mid-sized animals more, and adults most. This increase is effected in part, at all events, by the division of the yellow-brown cells (Pl. 28, fig. 18). When examined in the living state, soon after the capture of the animal, each yellow-brown cell is seen to contain many irregularly oval or polygonal, yellow-brown discs which occupy the greater part of the cell. Lying about the margin are a number of more elongated, browner, somewhat boat-shaped bodies. The remainder of the cell consists of one or more clear, transparent, vacuolar areas; occasionally, colourless, rounded refractive bodies also occur (Pl. 27, fig. 7).

But if the microscopic examination is made immediately after the animals have been caught, the yellow-brown cells are seen to differ markedly from those of animals kept for an hour or so before examination. Most of the yellow-brown cells in the just-caught specimens have a large colourless, transparent, anterior region, which may be as much as half

the size of the whole cell; in others, two or even three such clear vacuole-like structures may occur. Moreover, instead of the occasional refractive globules, the yellow-brown cells of animals examined immediately after capture are found to contain, in many instances, large numbers of such globules (Pl. 27, figs. 8 and 9). Not only are these globules to be observed in the yellow-brown cells, but they may be seen also lying in the animal tissue itself (Pl. 26, fig. 6).

The numerous yellow-brown discs which make up the bulk of the cell contain two pigments—a yellow pigment and a green pigment having the appearance of chlorophyll. The green is masked by the yellow pigment, but may be demonstrated by treating the animal with 90 per cent. alcohol. Thus acted on, the yellow pigment screening the chlorophyll dissolves rapidly and discs become green. Similarly, hot water destroys the yellow and reveals the green pigment. In this respect the yellow colouring-matter behaves like Phycophæin, the pigment of the brown algæ. When 90 per cent. alcohol is added to cells, the yellow pigment of which has been extracted by hot water, the green colour is intensified. The yellow pigment is only slightly soluble in dilute alcohol. Acted on by concentrated sulphuric acid the cells take on a beautiful emerald-green colour. These reactions serve to distinguish between the yellow-brown cells and the orange glands. The pigment of the latter is removed by 90 per cent. alcohol, but when so removed leaves no green colour behind. Treated with concentrated sulphuric acid the orange pigment of the glands undergoes no change.

There can be no doubt that the discs which make up so large a part of the yellow-brown cell and which give to it its distinctive colour are chloroplasts. The reason for the marked difference between the more central, polygonal, or oval, pale chloroplast, and the peripheral, elongated, boat-shaped, darker chloroplasts is not obvious. The contrast between the two kinds is so marked as to make it unlikely that the difference is merely optical—due to the latter presenting themselves in profile. It may be that the more peripheral

chloroplasts are somewhat affected by the presence of the cell in the animal tissue, and, shrunken somewhat, appear darker in colour (Pl. 27, fig. 8, and Pl. 28, figs. 11, 12, and 13), or it may be that the differences indicate a real dimorphism of the chloroplasts.

The wall of the yellow-brown cell is extremely delicate, and gives no staining reaction whereby its nature may be determined. It is not composed of cellulose. A central, spherical nucleus staining green with methyl-green acetic may be demonstrated in many of the cells. Where present it lies about the middle of the cell in a sheath of protoplasm from which radiating strands run toward the periphery (Pl. 28, fig. 18). In some preparations the nucleus, slung in the manner just described, appears to lie at the base of a colourless "neck" of protoplasm in the same position as that in which it occurs in a chlamydomonas cell. Occasionally two nuclei may be seen in a yellow-brown cell, at other times the cell appears to have divided, but division of the nucleus not to have taken place so that a nucleus occurs in one part, and not in the other (Pl. 28, fig. 18 *d*).

(*b*) The Photosynthetic Activity of the Yellow-brown Cells.—The structure of the yellow-brown cells of C. paradoxa points to the conclusion that these bodies are of the nature of algæ. Other evidence based on their origin and given in Section III confirms this conclusion.

Now if the yellow-brown cells are of the nature of algæ, and if, as shown, they divide and grow in the animal tissue, it is to be expected that they may exhibit photosynthetic activity. Unfortunately but little is known either of the nature of the substances photosynthesised by members of the different groups of the brown and yellow-brown algæ or of the form in which these substances are stored as reserves in the plant-cells.

According to Hansteen (1900) the reserve-substance formed from the product of photosynthesis in the Phæophyceæ (brown algæ) consists of refractive globules of a carbohydrate which he calls fucosan. Crato (1893) holds that the reserve-substance of the brown algæ is contained

in vacuole-like bodies termed physodes. The contents of the physodes blacken with osmic acid, and give the vanillin reaction for phloroglucin or tannin. According to Koch, (1896) who denies the presence of tannin, the physodes contain a colloidal substance formed from a polysaccharide and also a nitrogen-containing body.

In Dictyota, Hunger (1902) states that a carbohydrate occurs in the outer, and a glucoside in the inner, layers of cells, and that these substances disappear from the cells of dark-kept plants. The assimilate of the Diatomaceæ is stored generally outside the chloroplast in the form of oil, (Oltmanns, 1905). Dinobryon, a member of the Chrysomonadineæ, contains white balls of unknown nature termed by Klebs (1892) leukosin. In other members of this group Meyer (1897) describes a fatty oil as the reserve substance. The nature of the reserve-form of the assimilate of the Zooxanthellæ occurring in Radiolaria has been investigated by Haeckel, Müller, Brandt, and others, but the conclusions are not very convincing. According to Haeckel these yellow cells give a blue reaction with iodine (Cienkowski, 1871), whereas Müller obtains a brown reaction, deepening on the application of sulphuric acid. Brandt (1885), in his well-known monograph on the Radiolaria, states that the assimilate lies in the chloroplast (chlorophyll body), and is of two kinds. One form is starch, consisting of fine granules, which occur as a thin ring about a large vacuole. These bodies are not doubly refractive, and are described as hollow starch grains. The other form of assimilate consists of doubly refractive, fine, irregular, red-violet granules, which undergo no colour-change with iodine. They are not numerous in diffuse light, but increase in numbers when animals are exposed to bright light for half an hour.

With reference to these various observations several points may be noted. First, the iodine coloration, brown or blue, must not be taken to indicate the certain occurrence of starch. Yellow algal cells such as Zooxanthellæ are rich in carotin, which substance gives a green or blue-green colour

with iodine. Hence the colour-reaction obtained by Haeckel and Müller may well have been due to the presence of carotin, and not to the presence of starch.

Again, Brandt's hollow starch grains—fine granules surrounding a vacuole—suggest, though somewhat remotely, a degenerated pyrenoid with its starch sheath. Third, the red-violet granules described by Brandt cannot be regarded, merely because they increase in numbers in bright light, as of the nature of photosynthetic products. It is at least as likely that they owe their origin to a light-induced modification of carotin or some similar pigment.

In any case accurate information as to the nature of the assimilate of the Zooxanthellæ is lacking, and it is to be hoped that those engaged in researches on the Radiolaria will clear up this matter.

(*c*) The Reserve-fat of the Yellow-brown Cells.—The yellow-brown cells of C. paradoxa contain no starch; but reference has already been made to the occurrence, in the yellow-brown cells of animals examined immediately after capture, of refractive globules. These droplets (Pl. 27, fig. 8) lie apart from the chloroplasts in the colourless reticulum of the cell. The substance composing them is soluble in absolute alcohol, stains a grey or brownish colour with osmic acid, and gives the yellow-red fat-reaction with Sudan III. The osmic reaction is somewhat faint till after the preparation has been treated with alcohol, when the brown coloration of the globules gives place to black. This behaviour is, according to Bolles Lee ('Microtomist's Vade Mecum,' p. 36, 1900), characteristic of stearin and palmitin, and of the corresponding fatty acids. It may, therefore, be concluded that the refractive droplets of the yellow-brown cells consist of, or at least contain a fatty substance. That they are of the nature of reserves is suggested by the facts that they disappear gradually from the yellow-brown cells of animals kept under the somewhat abnormal conditions obtaining in the laboratory, and that this disappearance is more rapid in dark-kept animals.

For example, the yellow-brown cells of animals caught on July 24th, 1907, contained numerous large fat-globules. Animals from this catch were transferred to filtered sea-water, and kept, some in darkness, others in the light. After four days (July 28th) the dark-kept animals contained no fat, whilst those kept in the light still contained fat-globules in their yellow-brown cells.

It follows, therefore, that either the oil-globules are reserve substances formed from the products of the photosynthetic activity of the yellow-brown cells, or they are derived from the tissues of the animal.

So little is known of the modes of nutrition of the lower algæ that the à priori objection to the latter interpretation is valueless. For it is based implicitly on the hypothesis of autotrophic nutrition of the algæ in general.

Experiments designed to determine this question seem at first sight to support the conclusion that the fat is derived from the animal tissues. If animals, whose yellow-brown cells contain fat, are placed in darkness, some in filtered sea-water, others in ordinary sea-water with seaweed from the Paradoxa zone, it is found that the fat disappears more rapidly from the animals kept in filtered sea-water than from those supplied with food. From this it might be concluded that new supplies of fat reach the yellow-brown cells from the digested products of the food taken in by the animal. Against this, however, is the fact that the disappearance of the fat from the yellow-brown cells of animals kept in darkness and supplied with seaweed and hence with food is only a matter of time. Kept under such conditions, although the animals continue to feed, the fat disappears from the algal cells, and finally these cells undergo degeneration. The right inference to be drawn, therefore, from the slower disappearance of fat from the yellow-brown cells of dark-fed animals would seem to be that the food ingested by the animal acts in some measure as a protection to the yellow-brown cells hence the reserves of food-material accumulated in these cells are not drawn upon by the animal so rapidly as is the

case when no other food is available. What seems to be conclusive proof that the fat of the yellow-brown algal cells is a product of photosynthetic activity is the fact that when animals are placed in filtered water and so deprived of all food except that which reaches them from the algal cells and when the conditions for the nutrition of these cells are made as favourable as possible, e. g. by the addition of extra nitrogen in such form as potassium nitrate, then so long as the animals are exposed to light their yellow-brown cells continue to contain oil globules. Thus, in one experiment, animals were placed, some in filtered sea-water in the dark, others in filtered sea-water with extra nitrogen in the light. After fourteen days (August 21—September 5), though there was no fat in the algal cells of those kept in the dark, fat-globules were still present in the yellow-brown cells of the light-kept animals. It is therefore to be concluded that the fat-globules of the algal cells of C. paradoxa are reserve products of the photosynthetic activity of these cells.

It follows naturally from the foregoing experiments and conclusions that the fatty reserves of the yellow-brown cells are drawn on to supply material for the growth and metabolism of these cells. But there is evidence that the reserves of fat in the algal cells are also drawn upon and utilised by the animal tissues.

The general proof that the yellow-brown cells do pass on food substances to the animal tissues will be given later. That the reserve-fat of the algal cells is one of the substances transferred to the animal the following observations render probable :

In the first place, the tissues of animals whose yellow-brown cells are rich in reserve-fat also contain large numbers of globules of similar nature. These fat-globules, lying in cells of the animal tissue, have the same optical properties as, and give reactions similar to, those of the algal cells. In the second place, the appearance of the fat and its relation to the yellow-brown cells is, in freshly captured animals, most suggestive of secretion, recalling almost the appearance

of a cell of a mammary gland in its active stage. The large, clear, anterior end of the yellow-brown cell, only visible in fresh caught specimens which by the nature of their habitat have just been exposed to high light-intensity, is seen often to contain one large oily droplet. In some, one or more large droplets are situated in the deeper part of the clear anterior end, whilst in others a single large oil-drop lies close against its anterior margin, separated only from the animal tissue by the finest of membranes. Finally, other large globules may be seen lying just outside the colourless anterior borders of yellow-brown cells, and presenting every appearance of having been extruded from them (Pl. 27, figs. 8 and 9). It is not, of course, suggested that all the fat-globules, of which great numbers occur in the body of fresh-caught animals, are derived from the algal cells. Doubtless some fat comes from the digested solid food, diatoms, foraminifera, copepods, tetraspores, and the like on which C. paradoxa feeds so copiously. But it is claimed that the algal cells, when actively photosynthesising, regularly pass on the excess of their assimilate in the form of fat to the animal tissues.

These observations throw light on certain others which are recorded in the paper on "Convoluta roscoffensis" (Keeble and Gamble, 1907). Here, in some preparations, rows of fatty granules are to be seen passing from the green, algal cell to the neighbouring animal cells. It is highly probable that this fat represents the translocation-form of the ternary substance which C. roscoffensis obtains from its contained green cells. In this former paper, where proof was given that food-substances do actually pass from the green cells to the animal, it was suggested that the reserve-starch of the green cell travelled from that cell to the animal tissue in the form of sugar. But, having regard to the known fact that in plants starch is very readily converted into fat it may well be that the reserve-starch of the green chlamydomonadine cell of C. roscoffensis undergoes conversion into fat before passing out from the algal cell into the animal tissues.

(*d*) The Fate of the Yellow-brown Cells.—The yellow-brown cells persist throughout the normal life of C. paradoxa. Comparatively few in the very young, the number increases with the size of the animal. A casual observer, noting this large number of cells in the body of the adult, many of them of great size and many undergoing division, would be tempted to conclude that the yellow-brown cells were living parasitically upon the animal.

The evidence just given, together with that which follows, demonstrates that this way of looking at the relationship between C. paradoxa and its yellow-brown cells is erroneous. For, beside the tribute of fat which it exacts regularly from the algal cells, the animal has another and more drastic mode of exploiting them.

If animals are kept in darkness in sea-water filtered through a Pasteur-Chamberland filter, they exhibit, of course, starvation phenomena and become reduced greatly in size. This reduction in size is greater, and takes place much more rapidly in such dark-kept than in light-kept animals.

Thus animals of the same history were put in filtered sea-water on August 29th, 1907, and kept some in light and some in darkness. After nine days the light-kept animals were recognisably less reduced in size than the dark-kept. Measurements gave, in divisions of the Leitz ocular micrometer, oc. 2, obj. 3 (= L. 2. 3) $140 \times 60 = 8400$ in the case of a light-kept: $75 \times 44 = 3300$ in that of a dark-kept animal. That is the area of the former measured 142 sq. mm., the area of the latter 56 sq. mm., or $2\frac{1}{2}$ times less.

The experiment, which was repeated frequently in the course of this research, demonstrates that, in the light, supplies are forthcoming from yellow-brown cell to animal, and that, in the dark, such supplies are either altogether lacking or much smaller in amount. In other words, the experiment confirms the previous conclusion that the yellow-brown cells are the seat of photosynthetic activity, and that products of this activity pass from algal cell to animal tissue.

Animals kept for a long period in ordinary filtered sea-water, even though they are exposed to the light, become ultimately reduced almost to microscopic size. Thus, examples put in filtered water (July 24th, 1907) measured (L. 2. 3) $120 \times 83 = 9900$. After a month (August 23rd) a specimen measured $60 \times 30 = 1800$, i. e. $\frac{1}{5}$ of its original size. That such small animals as C. paradoxa are able to withstand starvation for such long periods points definitely to the conclusion that they are living at the expense of their algal cells. Microscopic examination of animals kept in light and darkness in filtered sea-water shows that, as the animal tissues waste, so the yellow-brown cells become more and more reduced both in size and numbers.

That this reduction of the algal cells is more rapid in "dark-filtered" than in "light-filtered" is seen in the results of the experiment already cited, in which, after nine days, the size of "light-filtered" animals was to that of "dark-filtered" as 2·5 : 1. At the time these measurements were taken, the records for the algal cells in the two cases of light-kept and dark-kept animals ran thus:—Light-filtered, number of yellow-brown cells double that in dark-filtered.

Size of yellow-brown cells:—Light-filtered, most 4 divisions (ocular micrometer, L. 2. 6) = 14·8 μ; some 6 div. = 22·2 μ and more. Dark-filtered, most 2 div. = 7·4 μ; some 3 div., some 5 div. = 18·5 μ.

This rapid degeneration of algal cells in animals kept in filtered sea-water in the dark offers some means of obtaining information as to the nutritive relations which exist between yellow-brown cells and animal. For the purpose of this enquiry, animals of similar origin were placed in the dark, some in filtered sea-water, others in unfiltered sea-water with weed rich in food-material (diatoms, etc.) from the Paradoxa zone. After nineteen days the number of yellow-brown cells was greatly reduced in both. It is true that the reduction in numbers and in size had proceeded further in the dark-unfiltered than in the weed-fed animal, and that, whereas no normal yellow-brown cells were to

be observed among the former, among the latter some few intact normal yellow-brown cells persisted. Since there is no apparent reason why the algal cells of animals supplied with proper food should suffer curtailment of supplies merely because they are placed in the dark it is reasonable to conclude that, whatever may be the contribution toward the raw material of the food which the yellow-brown cells receive from the animal, they do not receive elaborated material from that source. The somewhat slower reduction of algal cells in the fed animals indicates that, in the presence of food derived from the outside, the yellow-brown cells are in some small measure spared.

Taking all the facts together: from the behaviour of the animals and algal cells in filtered sea-water (light), and in filtered sea-water (dark), and in ordinary sea-water with weed (dark), it must be concluded that, in darkness, the algal cell failing to photosynthesise material for itself and unable to obtain supplies of elaborated food material from the animal, falls a victim to the digestive activity of the surrounding animal tissues. In its weakened state, brought about by starvation, it can no longer offer resistance to this digestive activity, whereas in its normal state it buys off this fate at the price of the tribute in kind, in the form of fat, which it pays continuously to the animal.

Histological examination of the dark-kept animals supplies evidence that degeneration of the algal cells is not a mere decay within the animal body, but is the result of a process of digestion exerted on them by the animal tissues. The first sign of this digestive process consist in a reduction in size and a more spherical shape of the yellow-brown cells. The chloroplasts become smaller and rounder, though the nucleus and the clear anterior end of the algal cell may persist. Each reduced algal cell may now be seen lying in a distinct digestive vacuole containing a pink fluid. The pigment of the chloroplasts is dissolved, and diffusing out of the cell may colour the vacuolar fluid brown. At this stage the chloroplasts are greenish, but later they become colour-

less. Finally, nothing appears to be left of the algal cells, but heaps of few or many colourless, curiously persistent, granules. The time required for the algal cells to be reduced to such granular remnants varies considerably; in some experiments it took fourteen days, in others nineteen days or longer.

Experiments were instituted in order to ascertain whether the remnants of the yellow-brown cells left in the body after prolonged exposure to darkness were capable of regenerating into normal yellow-brown cells; but, as an understanding of the results obtained requires a knowledge of the appearance of the algal cells in their young stages, the results are deferred till after this appearance has been described (p. 460). It may, however, be mentioned here that, when a redevelopment of algal cells was induced in animals which had been kept previously for long periods in darkness, the animals which, during their sojourn in darkness, had become reduced to the size of larvæ, began at once to grow again. Thus, after fourteen days darkness three animals measured superficially in divisions of the ocular micrometer L. 2. 3:— $77 \times 53 = 1{\cdot}18$ mm.; $71 \times 43 = {\cdot}87$ mm. and $48 \times 28 =$ ·37 mm. Average = ·8 mm. Whilst animals similarly treated but subsequently brought into the light and supplied with weed, under which conditions algal cells reappeared, measured, after four days exposure to the new conditions of light and food, $92 \times 55 = 1{\cdot}46$ mm.; $74 \times 54 = 1{\cdot}15$ mm.; $78 \times 40 = {\cdot}9$ mm. and $50 \times 30 = {\cdot}5$ mm. Average 1 mm.

Though, by reason of original differences in size, too much weight cannot be given to individual measurements, the phenomenon of apparent increase was so uniform as to admit of the conclusion that growth of the animal is bound up with the presence of living yellow-brown cells.

(*e*) The Origin of the Yellow-brown Cells.—A larval Convoluta paradoxa, as it hatches out from its egg-capsule, contains no yellow-brown cells nor any precursors thereof. C. paradoxa is in this respect precisely similar to

C. roscoffensis, which, as has been shown, is at birth altogether free from algal cells. That C. paradoxa hatches as an uninfected larva is easily shown; indeed, whereas in C. roscoffensis the difficulty is to prevent infection by the algal cells, in C. paradoxa the difficulty is to induce it.

If the egg-capsules of C. paradoxa are placed in ordinary sea-water, without any weed from the Paradoxa zone, the animals do not become infected. They feed on diatoms, etc., which may be present in the water, yet not only do they not increase in size, but rapidly become smaller, and fail altogether to show signs of development. Thus, a series of measurements of just-hatched animals gave length by breadth in divisions of ocular micrometer L. 2. 3 :—3500, 3600, 4500, 5725, or in sq. mm.: 1·02, 1·04, 1·3, and 1·6; whereas a series of measurements of uninfected animals of various ages all put with water rich in diatoms and other food-material gave 1600, 1920, 2400, 2720, 3600, 4125, 4750, or in sq. mm.: ·5, ·56, ·69, ·78, 1·05, 1·2, and 1·4. That is, whereas the average size of the just-hatched animal is about 1·2 sq. mm., the average size of 7 animals supplied with food but not with the infecting organism is ·88 sq. mm.; and this in spite of the fact that the food-material (diatoms, etc.) is taken up readily enough.

In order to induce the development of the yellow-brown cells in the larvæ it is not sufficient to cause them to hatch out in water from the Paradoxa zone. Weed from the zone must be provided, and the supplies of this weed replenished several times during the first few days after hatching. Even so, infection does not follow inevitably. In cases where, by the adoption of this procedure, infection ensued, it was found to occur very rapidly.

For example, in one instance, animals were caught on August 12th; on the 14th eggs were found on the small pieces of weed left with them. The young, hatched on the 16th, were put in a separate vessel with water and fresh weed from the Paradoxa zone. On the 17th the specimens examined showed no signs of yellow-brown cells. Another piece of

weed from the Paradoxa zone was added, and on the following day infection was observed. The results of this experiment are summarised in Table I (Infection Record), where it will be seen that, of 14 animals examined, 8 showed no sign of infection, and 6 showed infection in various stages: from the stage in which one yellow-brown algal cell was present in the animal to that in which large numbers of yellow-brown cells occurred. This was one of the most successful of the many infection-experiments which were carried on in 1906 and 1907. In others the proportion of infected to uninfected animals was smaller, and in some cases no infection was obtained. From this it is to be concluded that the changed conditions in the laboratory affect the infecting organism adversely or that its distribution in the weed is sporadic, Yet another possibility must not be lost sight of, namely, that under some circumstances, e. g. when the conditions, as, for example, those obtaining in the laboratory, are somewhat unfavourable, the yellow-brown cells taken up by the larvæ may suffer digestion just as was shown to be the case with the yellow-brown cells of the adult. As indicative of the probability that this fate may overtake the infecting algal cell, several records occur in the notes of infection experiments to the effect that a larva, exhibiting no normal yellow-brown cells, nevertheless contained digested remnants of such cells. The fact that in nature no C. paradoxa are to be met with which do not contain large numbers of yellow-brown cells is no argument against the probability of this view, since, under natural conditions, reinfection would doubtless occur. On the other hand, the conception that it is not a foregone conclusion that, once arrived in the body of C. paradoxa, the algal cell will grow and divide, contributes a little toward an understanding of the way in which the present relations between algal cell and animal may have originated. The animal, it may be conceived, plays with respect to the yellow-brown cells the part which that discriminating Providence, Natural Selection, plays with respect to living things in general. Not all the entering algal cells pass its test; nor,

indeed, all those belonging to the chosen race of yellow-brown algæ, but only those that can withstand digestion. How some possess this power, or acquire it, may likewise be imagined. When, as often happens, the body of C. paradoxa is gorged with solid food—in one case upwards of 70 diatoms were counted in a single animal—the activity directed toward the digestion of any single cell is likely to be reduced. Thus, it may be that the glut of solid or reserve food in the body may act temporarily as a protection to the yellow-brown cells. Profiting by this respite, the algal cells may react adaptively by change in osmotic power, or in permeability of plasmatic membrane, and so prepare to resist the action of the normal digestive juice of the animal. By what chemical mechanism the animal cells come to tolerate the presence of these foreign cells, or by what means the fatty reserves are discharged from the algal cells, it is impossible to say. An investigation of the proteases and other enzymes of C. paradoxa, particularly with a view to determine in what medium they act, might throw some light on the enigma of what maintains the balance between foreign algal cell and animal so equipoised that the former behaves like an integral part of the latter. To discuss such questions in the present state of our knowledge of invertebrate physiology, though tempting, is vain.

Apart, then, from all theoretical considerations, experimental proof is forthcoming that the eggs of C. paradoxa contain no yellow-brown cells; that larvæ, unless exposed to the weed of the Paradoxa zone, remain uninfected, and that infection may be induced by bringing the recently hatched animals into contact with this weed. Thus, except for the fact that it is much more uncertain in C. paradoxa, there is with respect to infection a complete agreement between C. paradoxa and C. roscoffensis. Both animals require to be reinfected in each succeeding generation. In neither is there any transmission of infecting organism from one generation to the next.

The greater certainty of infection of C. roscoffensis

under experimental conditions is, as has been shown (Keeble and Gamble, 1907), in part due to the regular and close relation which exists between the infecting organism in its free state and the egg-capsules of the animal and in part to the ubiquity of the infecting organism. In that species the free, four-flagellated, Chlamydomonadine alga which constitutes the infecting organism settles habitually on the gelatinous egg-capsules, and, undergoing division, gives rise in the empty capsules to spherical colonies. It was the observation of this fact which led to the isolation of the infecting organism of C. roscoffensis.

In C. paradoxa this habit on the part of the free infecting organism of frequenting the egg-capsules does not appear to obtain, or, if it obtains, is much more difficult of demonstration.

Repeated experiments were made in which the capsule-remnants, left after the larvæ had escaped, were put in filtered sea-water and kept under observation; but, though these capsules developed a fairly luxuriant flora of diatoms, brown flagellates and yellow-brown cells resembling in number of chloroplasts and in refractive inclusions the yellow-brown cells of the adult C. paradoxa, it was not found possible to induce the infection of larval animals by putting them in contact with these possible sources of infection. Too much weight must not, however, be attached to these negative results; for the experimental difficulties are greater in the case of C. paradoxa than in the case of C. roscoffensis, partly because the former animals are more difficult to obtain in any considerable numbers, and partly because of the difficulty of imitating in the laboratory the conditions under which C. paradoxa lives in the open.

For the same reasons all attempts to isolate the infecting organisms or to obtain it in its free stage have failed. The yellow-brown cells of the adult or young infected animal cannot be induced to grow when removed from the body of the animal. The same fact has been established with respect to the green algal cells of C. roscoffensis. They also fail

to develop when removed from the body. There can be little doubt that this failure on the part of the green cells of C. roscoffensis to maintain themselves when separated from the animal is due to the fact that they undergo whilst in the animal body partial nuclear degeneration. The same is probably true of the yellow-brown algal cells of C. paradoxa. It being impossible to isolate viable yellow-brown cells from the animal the only alternative is to seek among the weed for a corresponding form, to cultivate it, and apply the infection test. Though yellow-brown cells, resembling in the most striking way those of the animal, are to be met with occasionally, especially in the capsule flora (Plate 28, fig. 17), and though in one instance a brown cell with elongated anterior end very suggestive of a flagellated organism was observed on an egg-capsule (Plate 28, fig. 16), the isolation of the free alga which, when in the animal, gives rise to the yellow-brown cell, has not yet been accomplished.

The earliest stage yet observed shows the body of the animal to contain a single algal cell (Plate 28, figs. 10—15). The size of this cell varies considerably in the different cases in which it has been seen. In one example the cell was spherical, 7·4 μ in size, with greenish-yellow chloroplasts. In another it was of oval shape, measured 16·5 μ, and exhibited, like the yellow-brown cells of fully infected animals, but in a more marked manner, a peripheral series of flattened yellow chloroplasts with a number of pale grey-yellow chloroplasts occupying the body of the cell. In yet other cases, in which more than one cell was present, the infecting alga exhibited a large, clear, transparent region, a group of flattened brown chloroplasts pointed at either end pressed against the wall of the cell, and a number (8—16) of pale yellow structures more like daughter-cells than mere chloroplasts. This appearance was strikingly exhibited in one case, where the animal contained five algal cells, of which the largest was 37 μ × 37 μ, the others 16—18 μ. The large cell was surrounded by transparent thickish wall, against one side of which the elongated, dark brown chloro-

plasts were pressed, whilst in the centre a large number (thirteen counted) of irregular, oval, pale yellow-brown bodies occurred (Pl. 28, fig. 15 *a*, *b*, *c*). These yellow-brown structures contained each a central dark spot, and each when seen in profile showed the appearance of a vertical cleft extending through about half its depth. The wall by which these yellow-brown bodies were enclosed underwent change of shape due to the movement of the animal, whence it may be assumed that it consisted of animal vacuole-wall together with extremely delicate algal cell-wall. During examination the vacuole burst, and the contained irregularly oval, pale brown bodies were discharged into the body, and lay in groups of twos and fours. None of the other vacuoles of the body burst, and so there is a possibility that what was witnessed was the liberation of a group of daughter-cells from a mother-cell. On this view the peripheral chloroplasts are to be regarded, as already suggested, as the partially degenerate chloroplasts of this mother cell and the yellow-brown irregularly oval bodies as daughter cells, and not merely as chloroplasts. This interpretation of the phenomena finds support from facts connected with the development of yellow-brown cells in the body both of the larval and adult animal. Thus, in the former, besides the large cells described, just infected animals not infrequently contain minute yellow or yellow-green cells with only three peripherally placed chloroplasts, one oval and two somewhat elongated and pointed (Pl. 28, fig. 12). In the adult animal also, beside the large yellow-brown cells with numerous chloroplasts, there occur minute yellow cells, consisting now of a single, now of several chloroplasts.

Figures, Pl. 28, figs. 11, 12, 13, 18, represent these various stages in the development of the yellow-brown cells of larval and adult animals. The paleness of the colour—greenish-yellow, grey-yellow, yellow—both of the small cells of the adult, and of the irregular, oval structures contained in the large, developing cells of just-infected animals supports the view that the infecting organism has a colourless, saprophytic, free stage as well as a yellow-brown, holophytic, free

stage, and that infection may arise from the former stage. The more normal, yellow-brown appearance presented by other algal cells which occur in just-infected animals likewise suggest that the alga may also be ingested in its holophytic stage. Like variations in appearance are presented by the infecting organism of C. roscoffensis, in its earliest stage in the body of that animal; and in this case it has been demonstrated that these variations in the earliest recognisable condition of the infecting organism are due to the fact that the latter may be ingested at almost any stage of its life-history, viz. as a green resting-spore, as a colourless resting-spore, as a green or colourless, non-motile daughter-cell, or as a four-flagellated, green, typically chlamydomonadine cell: all of which stages have been observed in the life history of the infecting organism of C. roscoffensis. Once within the body of C. paradoxa the yellow-brown cell develops rapidly, the daughter-cells to which it gives rise are sown about the body, and undergo further growth and division. By this means, and also possibly by formation of spores, the yellow-brown cells of the body, now to be numbered by hundreds, impart, together with the orange glands, the characteristic colour to the animal.

To return to the question of the reappearance of normal yellow-brown cells in animals which, after long exposure to darkness in filtered sea-water, are brought into contact with weed of the Paradoxa zone (p. 454).

The appearance presented by the new crop of algal cells, the smallness of their numbers, their large size and their resemblance to those of just-infected, larval animals, their occurrence side by side with colourless heaps of undigested granular remnants of the destroyed earlier crop of yellow-brown cells, all suggest that reinfection has taken place. And the fact that, when reinfection takes place, growth of the animal is resumed indicates how intimate has become this relation between alga and animal.

(*f*) The Nature of the Infecting Alga.—Pending the discovery of the free stage in the life-history of the yellow-

brown alga, nothing of certain value can be said as to the systemic position of this organism. It is evidently distinct from the Zooxanthellæ of Radiolarians. For, whereas these yellow-brown cells possess two chloroplasts, the infecting alga of C. paradoxa possesses, in its fully-developed state, a large number (ten or more); and, whereas the reserves of Zooxanthellæ consist of starch or some undetermined substance, those of the infecting alga of C. paradoxa consist of fatty globules.

The pigments of the algal cells consist of a yellow-brown substance soluble in water and probably similar to Phycochrysin, a pigment described by Gaidukov as occurring together with a chlorophyll-like, green pigment in Chromulina rosanoffii a member of the Chrysomonadineæ.

The brilliant emerald-green reaction given by the yellow-brown cells when treated with strong sulphuric acid is stated by Zimmermann to be given also by Diatomin, the brown pigment of the Diatomaceæ. The fact that the pigments of the Chrysomonadineæ are held to be allied to those of the diatoms gives some slight indication that perhaps the yellow-brown alga of C. paradoxa may prove to be allied to the Chrysomonadineæ rather than to the Cryptomonadineæ, to which group the Zooxanthellæ of Radiolaria have been referred; but the evidence is too slender to admit of more than a conjecture.

(*g*) The Significance of the Relation between Animal and Yellow-brown Cell.—The facts set forth in the preceding pages have demonstrated the intimacy of the relation between C. paradoxa and its yellow-brown cells. So dependent is the animal on these cells that, apart from them, it is incapable of growth or development. Larvæ which escape infection, although they take up solid food, fail to develop, decrease steadily in size and die. In like manner the algal cell, once in the body of C. paradoxa, becomes an integral part of that body, and is no more capable of independent existence than is any somatic cell of the animal. The yellow-brown cell stands to the animal cells in the same rela-

tion as a chloroplast-containing cell of one of the higher plants stands to the colourless cells of that plant. The parallel is even closer than might seem from the foregoing for, as will be shown immediately, just as the chloroplast-containing cell of a plant receives unelaborated food-material from the colourless cells and, in return, provides them with organic food substances, so the yellow-brown cell receives from the cells of the animal the raw material from which it elaborates organic food substances, and in exchange passes on organic food substances to the animal tissues. As was pointed out in a previous paper (Keeble and Gamble, 1907), a precisely similar relation holds between green Chlamydomonadine cell and C. roscoffensis. Viewed from the standpoints of the animals, C. roscoffensis and C. paradoxa, the relations between them and their respective algal cells admit of accurate definition. C. roscoffensis and C. paradoxa are obligate parasites. In the absence of their respective infecting organisms these species would become extinct.

But if the standpoint is shifted so that the relation is regarded from the point of view, so to speak, of the yellow-brown cell, the definition of this relationship will vary according as attention is directed to the individual yellow-brown cell or to the species of which that cell is a member. To the species, the relation is a meaningless episode; to the individual, ingested, yellow-brown cell, it is a great event. All that ingestion means to the species is that a certain, probably small, proportion of its members meet their fate in the body of C. paradoxa. It is only another hazard in the struggle for existence. The algal cells which are incorporated into the tissues of C. paradoxa never escape and so never impress the species with the consequences of that event; any modification which the algal cells may undergo as a result of their sojourn in the animal body can leave no mark on the species.

To the species C. paradoxa, infection or non-infection are matters of life or death; to the species "infecting organism,"

ingestion means only a somewhat increased death-rate. Hence, whilst to the infecting organism the effects of the relation are nil, these effects are permanently recorded in changed habit and modified development in the animal. Although to the species "infecting organism" it means so little, to the individual ingested algal cell incorporation in the tissue of the animal means much. The rapid growth and multiplication of the ingested algal cells indicate that the tissues of the animal offer a medium highly favourable to the development of those cells. So striking is this, that observers not infrequently infer from it that the alga is living parasitically in the animal body. Though this has been shown in the preceding pages not to be the case, the problem remains: to what peculiar conditions obtaining in the tissues of the animal is due this luxuriant vegetative development of the algal cell?

It was suggested, when this same problem was under consideration in the case of C. roscoffensis, that the luxuriant growth of the ingested algal cells was due to the favourable position which they occupy with respect to nitrogen. It is known that the amount of nitrogen present in sea-water in a form available to plants for synthetic purposes is extremely low; so low that it is highly probable that the amount of nitrogen is the factor which limits the development of marine plants and animals. Thus, according to Johnstone (1907), who bases his calculations on Raben's estimates, "the amount of nitrogen-compounds in Baltic and North Sea water may be taken as about ·2 mgr. in a litre, or 2 parts in one million." Whence it follows that the infecting organism, living free in the sea, must be hard put to it, in common with its competitors, to obtain sufficient nitrogen. Once in the body of C. paradoxa the state of affairs, so far as nitrogen is concerned, is changed. Since this animal possesses no excretory system, its waste nitrogen, stored within the body, is at the disposal of the alga. As von Graff has suggested acutely (1891), the transverse bands of refractive substance, white by

reflected, black-brown by transmitted light, which occur in the body of C. paradoxa consist probably of urates (Pl. 26, figs. 4 and 5). The granular substance of which these bands are composed is always present, although the amount varies very considerably. Even in the just-hatched larva the bands are indicated as grey-black patches formed by groups of sparse granules. As the animals mature the amount of granular substance increases, though at the time of egg-laying it may disappear almost entirely. Now the hypothesis that the yellow-brown cells may utilise uric acid for their proteid-synthesis presents no difficulty. For instance, it has been established that holoplytic plants may utilise for this purpose organic nitrogen in many different forms, e.g. urea, uric acid, asparagin, leucin, tyrosin, guanin, kreatin, hippuric acid, etc., and, moreover, it has been shown that uric acid may replace nitrates in water-cultures of the higher green plants (Pfeffer, 1900).

The problem of the luxuriant development of the yellow-brown cells in the body of C. paradoxa may be thus stated in terms of the nitrogen-hypothesis:—C. paradoxa contains stores of waste organic nitrogen, presumably in the form of urates. Such substances are known to serve as sources of nitrogen to various plants. Is the yellow-brown cell able to utilise such substances?

In order to answer this question two modes of experimentations were adopted. The first method was as follows:—Batches of animals of similar sizes and origin were kept for some time in filtered sea-water with uric acid, and their condition compared with that of control animals. In one experiment of this kind of animals which had been kept in darkness with weed for nineteen days and which had undergone considerable reduction in size and in the number of their contained yellow-brown cells, some were put in filtered sea-water with uric acid, others were left with weed in the dark. After three days (September 9th—12th) the animals which had remained with weed in the dark were very colourless, and had lost most of their yellow-brown cells;

whilst those exposed to the light and supplied with uric acid were pale brown in colour, and were seen, on microscopic examination, to contain a very considerably larger number of intact yellow-brown cells.

Another experiment was started on August 28th with three similar lots of animals. These lots may be designated "filtered, light," "filtered, light, uric," and "filtered, dark." After twenty-one days (September 18th) the reductions in size of the animals and in the numbers of their yellow-brown cells were very great in the "filtered, dark," and least in the "filtered, light, uric." The measurements of animals taken as samples were—"filtered, dark" 55 × 20 divisions (oc. micrometer, L. 2. 3); "filtered, light, uric" 90 × 40. That is the animals in the light with uric acid were more than three times as large as those in filtered water in the dark.

The experiment was continued. During the following weeks the "filtered, dark" and "filtered, light" animals dwindled, lost all their yellow-brown cells, became of microscopic size, and finally died. On October 21st specimens in "filtered, light, uric" were still alive, of recognisably brown colour, and possessed of many normal, yellow-brown cells. Finally, on October 28th a "filtered, light, uric" specimen was still alive, and, though reduced considerably in size, was rich in yellow-brown cells showing no sign of degeneration or of digestion.

When it is remembered that the animals were kept in limited supplies of sea-water only occasionally renewed, and that, during the time of the experiment, they were brought from Brittany to Reading, the effect of the uric acid in maintaining the yellow-brown cells and the animals alive for the space of two months will appear the more remarkable. From the experiment it is clear that the yellow-brown cells utilise uric acid as a source of nitrogen. It follows also that when the conditions admit of photosynthesis and when uric acid is supplied as a source of nitrogen the yellow-brown cells not only maintain their own existence, but also that of the animal.

That the algal cells may contribute not only fatty but also

proteid material to the animal, the results of the second mode of experimentation render highly probable.

Here, in lieu of determining the effect of uric acid on the life of algal cell and animal, its influence on egg-laying was investigated. The results of these experiments are summarised in Table II. In the first experiment of this series (started August 21st), five lots, each of twelve animals, were placed under the conditions indicated in columns 1—5 of the Table.

As the numbers in these columns show, but one clutch of eggs was laid in "filtered sea-water, light," one in "filtered sea-water, dark," none in "dark, weed" (column 5), four in "light, weed" (column 3), and six in "filtered, light, uric, and potassium nitrate" (column 2).

The next experiment (August 28th) shows that uric acid alone suffices to induce the production of eggs. For, whereas in "filtered sea-water, light" only eight clutches (column 1), and these containing but few eggs, were produced by fifty animals; seventeen clutches (column 2), with larger numbers of eggs, were laid by an equal number of animals kept in filtered sea-water in the light with uric acid.

The third experiment shows that nitrogen in the form of potassium nitrate is also efficacious in inducing egg-laying (Table II, 2*b*).

The total number of egg-clutches produced in these three experiments are:—in "filtered sea-water, light," 9; in "filtered sea-water, light, and added nitrogen" (uric acid, potassium nitrate, or both), 27.

These results would appear to prove that during the later stages of egg-development, when reserves are finding their way from animal to egg, a considerable portion of these reserves is obtained by the animal from the yellow-brown cells. The small number of egg-clutches laid in filtered water in the light indicates that the reserves of the animal tissues do not suffice to supply the eggs with material for their full development. It is noteworthy in this connection that, as before stated, the granular substance of the refrac-

tive bands undergoes marked reduction in amount, and frequently almost entirely disappears at the time of egg-laying. The stimulatory action of added nitrogen on egg-production may be, and probably is, due to the transmission from the algal cell to the animal, not only of the fatty assimilate, but also of products of proteid synthesis.

It is possible, however, that this increase in egg-production is an indirect rather than a direct consequence of added nitrogen. It is known from Boussingault's classical researches (1860) that nitrogen-starvation reduces enormously the photo-synthetic activity of the green plant. So here it may be that the poor results in "filtered sea-water, light," and the good results of "filtered sea-water and added nitrogen, light" are due, the former to the curtailment of fat-synthesis by the algal cell in the nitrogen-starved animal; the latter to the active fat-synthesis by the algal cell well supplied with nitrogen. However this may be, the two sets of experiments just described serve to account for the luxuriant development of the yellow-brown cells within the body of C. paradoxa. In their free state these algæ, like all marine plants, run grave and frequent risks of nitrogen-starvation or, at all events, have their increase limited by the shortage of available nitrogen. Wherever there is any leakage of nitrogen in any form—and traces of combined nitrogen must be given off from all such animals as the Acœlous Turbellaria—it is to be presumed that marine, motile plants will congregate. Congregating about C. paradoxa they will be ingested, and if, as happens in the case of the yellow-brown cell, they withstand digestion, they find rich stores of nitrogen—the waste nitrogen of the animal's metabolism—at their disposal. Thus they solve the problem of how to obtain sufficient nitrogen. To the individual infecting organism it is a solution; to the species it is none, for in effecting the solution to this nitrogen-problem the yellow-brown cell dooms itself to death without issue. It is to be noted that the association between algal cell and animal, though it has precisely the same significance in C. roscoffensis as in C. paradoxa, has gone a

step further in the former species. For, unlike C. paradoxa which continues all its life to feed voraciously, C. roscoffensis ceases very early to ingest solid food.

It is also noteworthy that the interpretation of the relation between animal and algal cell here offered throws light on certain facts concerning the distribution of algal cells in the bodies of various marine animals. The analyses of Natterer and Raben have demonstrated that the amount of combined nitrogen present in sea-water is less during the warm months, e. g. August, than during the cold months of the year, and that it is less in the warmer seas (Mediterranean) than in the colder seas (Baltic and North Sea) (Johnstone, 1907).

Now, as stated in the paper on C. roscoffensis, certain animals possess green or brown algal cells in one part of their range of distribution, but lack them in other stations. "Thus Noctiluca is colourless in the North Atlantic, and green in the Indian Ocean. British Alcyonium have no Zoochlorellæ, whereas the closely allied A. ceylonicum possesses them. It seems probable, indeed, that the maximum development of these associations occurs in the warmer seas." It would seem also probable that this parallelism between presence of algal cells and poverty of nitrogen is no coincidence, but that the former is causally connected with the latter.

The paleness of the colour of the infecting organism in its earliest stage of existence within the body of C. paradoxa suggests that this organism has in its free stage both holophytic and saprophytic phases. The similar behaviour of the infecting organism of C. roscoffensis has also been established. In its earliest stage within the body of the animal it may be altogether colourless. Such alternative phases, holophytic and saprophytic, pigmented or colourless, are known to occur in the life-histories of various of the lower organisms; for example, Diatoms (Nitzschia), certain Chlamydomonadineæ and Flagellates (Euglena). It is stated that the colourless phase may be induced by increasing the amount of soluble carbohydrate in the culture medium, or, in Nitzschia, according to Karsten, by augmenting the supply of organic

material (Czapek, 1905). But in the cases of the infecting cells of C. paradoxa and C. roscoffensis the development of assimilatory pigment appears to be associated with the increase in the amount of available nitrogen. And if this is the case it would seem probable that the colourless phase is brought about, not by excess, but by lack, of nitrogen. The suggestion is worth hazarding that the colourless saprophytic stages of such organisms as Diatoms, Euglena, etc., is a symptom of nitrogen-hunger. This hypothesis is at least as probable as that now prevalent. For, failing adequate supplies of nitrogen, no amount of carbohydrate-photosynthesis will avail; indeed, the more the carbohydrate-photosynthesis, involving, as it must do, the wearing out and reconstruction of the nitrogen-containing chlorophyll-machinery of pigment and plastid, the acuter will be the nitrogen-hunger; whilst, on the contrary, a shutting down of the photosynthetic process will economise nitrogen, and so postpone the evil day of nitrogen-starvation. Though the facts are not yet available for a confident statement, the hypothesis may be proposed that saprophytism generally may depend for its inception on nitrogen-hunger. It is tempting to push this provisional hypothesis to its limits, and to imagine that the great saprophytic group of the Fungi owes its origin to the changed mode of nutrition enforced upon it by lack of nitrogen. Lacking nitrogen, the photosynthetic activity of a green cell is greatly reduced or brought to a standstill; the chlorophyll machinery ceases to be worth its upkeep, and, wearing out, is too costly in nitrogen to be replaced. The organism will obtain directly from its environment as much carbon as is of use to it, together with as much nitrogen as it can get. It becomes a saprophyte.

Should this hypothesis be established, C. paradoxa and C. roscoffensis will rank high in interest among animals as suggesting the route along which far reaching evolution has travelled.

General Conclusions.

1. C. paradoxa occurs within a narrow belt of seaweed on the shore. It exhibits tidal migrations within this belt. The migratory movements are the resultant reactions to the various directive stimuli to which, in its changing environment, it is subject.

2. The egg laying and hatching are periodic. The periods synchronise with those of the neap-tides.

3. The eggs and just-hatched larvæ contain no yellow-brown cells. Preserved from infection, e.g., by hatching and maintaining in filtered sea-water, C. paradoxa remains free from yellow-brown cells (G. & K.).

4. By bringing uninfected larvæ in contact with seaweed from the Paradoxa zone infection is induced (G. & K.).

5. The infecting organism is an alga different from Zooxanthella of Radiolarians; its free stage is unknown. In the ingested state it is characterised by many chloroplasts, a colourless anterior end, and by the possession of fat globules in its colourless protoplasm.

6. Once introduced into the body of C. paradoxa the infecting organism multiplies rapidly.

7. The fat-globules of the algal cell are food-reserves. They arise as the result of the photosynthetic activity of the algal cells.

8. The reserve-fat of the algal cells is translocated from those cells to the animal tissues, and serves these tissues as food-material.

9. The ingested, yellow-brown, algal cell becomes, physiologically, an integral part of the animal, contributing towards its nutrition, and incapable of a separate existence.

10. The yellow-brown algal cells are indispensable to the animal. Uninfected animals fail to develop.

11. Nevertheless, starved animals digest their algal cells till no trace of these cells remains. Such disinfected animals

are capable of re-infection. With re-infection the growth of the animal is resumed.

12. The yellow-brown cells utilize in their constructive metabolism the waste products of the nitrogen-metabolism of the animal. The waste nitrogen of the animal is not excreted but is stored in the body, probably in the form of urates.

13. Animals deprived of solid food, but kept in the light in filtered sea-water to which uric acid is added, conserve their yellow-brown cells and maintain their lives longer than do animals not supplied with uric acid.

14. Similarly animals provided with uric acid lay many more eggs than are laid by animals kept under precisely similar conditions, but not supplied with uric acid.

15. The interpretation of the relation between yellow-brown cell and animal depends on the point of view:

From that of the animal, it is a case of obligate parasitism.

From that of the species "infecting organism," it is an insignificant episode, involving the loss of that, probably small, proportion of its members which are ingested.

From that of the individual ingested yellow-brown cell it is a solution of the nitrogen problem—a successful method of obtaining large supplies of nitrogen.

TABLE I.—Infection Record. Trégastel, 1907. Catch 12th; eggs laid 14th; hatched 16th (with weed of Paradoxa zone).

Date.	No. of infected animals.	No. of algal cells.	No. of uninfected animals.
17th	—	—	Several
18th	3	1 small algal cell. 1 large algal cell. 5 algal cells, 2 large, 3 small.	2
19th	—	—	2
21st	1	24 algal cells.	
22nd	2	Numerous.	2
30th	—	—	1
31st	—	—	1
Totals	6	—	8

TABLE II.—Egg-laying under various Nutritive Conditions. Convoluta paradoxa. Trégastel, 1907.

		1. Filtered sea-water.	2. Filtered sea-water + uric acid + KNO_3.	3. Unfiltered sea-water + seaweed from Paradoxa zone.	4. Filtered sea-water.	5. Unfiltered sea-water + seaweed from Paradoxa zone.
		LIGHT.			DARK.	
August 21.—Of animals caught same day, 12 placed under each of the conditions: 1—5.						
Examined	Aug. 25	1 clutch of eggs	5 clutches	0	1	0
,,	,, 27	0 ,, ,,	1 ,,	4	0	0
August 28.—Of animals caught same day, 50 placed under each of the conditions: 1 and 2*a*.						
Examined	Aug. 31	0 ,, ,,	7 ,, (Filtered sea-water + uric acid only = 2*a*)			
,,	Sept. 4	4 ,, ,,	8 ,, ,,			
,,	,, 7	4 (few eggs in these clutches)	2 ,, ,,			
August 29.—12 animals placed under each of the conditions: 1, 2*b*.						
Examined	Sept. 2	0 clutch of eggs	4 ,, (2*b*)	(Filtered sea-water + KNO_3 2 = 2*b*)		
Total egg-clutches laid under conditions 1 and 2 . .		9	27			

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EXPLANATION OF PLATES 26—28,

Illustrating Mr. Frederick Keeble's paper on "The Yellow-Brown Cells of Convoluta paradoxa."

PLATE 26.

Fig. 1.—Weed from Paradoxa zone with C. paradoxa (× 3). Egg capsules laid on weed seen as orange dots. The large weed Pycnophycus; the small weeds Ceramium and Rhodomela.

Fig. 2.—Egg capsule (× 40) with orange-pigmented eggs attached to weed of Paradoxa zone.

Fig. 3.—Egg (× 300) showing the pigment bodies irregular and dumb-bell shaped; but no yellow-brown cell.

Fig. 4.—Young C. paradoxa (× 70) two days after hatching. Body gorged with diatoms, etc., and containing one yellow-brown cell. The chief excretory band, already indicated, running transversely, a little distance behind the mouth.

Fig. 5.—C. paradoxa (× 50) showing the yellow-brown cells and the concrement granules (black by transmitted light).

Fig. 6.—The yellow-brown cells (× 70) as seen through the epidermal tissues in the living state. The orange glandular structures are also visible.

PLATE 27.

Fig. 7.—The yellow-brown cells (L. 2, $\frac{1}{10}$) as seen when the animal is examined soon after capture.

Fig. 8.—The cells as seen when the animal is examined immediately after capture. The clear anterior end of the yellow-brown cells is well marked, whereas in Fig. 7 it is not generally visible; and the cells, as well as the animal tissues, contain numbers of fat globules which are absent or very scarce in Fig. 7.

Fig. 9.—Yellow-brown cells showing fat undergoing excretion from their clear anterior ends.

PLATE 28.

Fig. 10.—C. paradoxa (× 20). (*a*) Mature female with eggs; (*b*) mature male; (*c*) immature animal seen from dorsal, and (*d*) from ventral surface. The bands and masses of excretory substance (concrement granules) appear

white by reflected light. In the mature female the amount of excretory substance is considerably less than in the male and immature stages.

Figs. 11, 12, 13.—Yellow-brown cells (× 330) as seen in just infected animals. In Fig. 11 the infecting cell has many chloroplasts differentiated into elongated, "boat-shaped," and polygonal. In Fig. 13 only three chloroplasts occur, but the differentiation into the two forms is already indicated.

Fig. 14.—Young C. paradoxa (× 45) 1—2 days after hatching; containing one large infecting cell and numerous colourless vacuoles.

Fig. 15.—*a*. The infecting cell of Fig. 14 enlarged (L. 2, $\frac{1}{10}$), showing peripheral, elongated, brown chloroplasts, and more central group of yellow, polygonal bodies, more suggestive of spores than of chloroplasts. *b*. The same cell undergoing changes of shape, due to the movements of the animal. *c*. The contents of (*b*) liberated in the body of the animal.

Fig. 16.—Egg capsules of C. paradoxa showing yellow-brown cells, one with pointed clear anterior end attached to the egg membrane.

Fig. 17.—Yellow-brown cells identical with those of the infecting alga occurring in the remnants of the egg capsule of C. paradoxa.

Fig. 18.—*a*, *b*, *c*, *d*. Yellow-brown cells showing nucleus. *e*. Minute yellow-brown cells showing stages of development from *e*, with single chloroplast to e_2 with several and e_3 with numerous chloroplasts (L. 8, $\frac{1}{10}$).

On the Diplochorda.

By

A. T. Masterman, M.A., D.Sc.

With Plate 29.

Part V.—Certain Points in the Structure of Tornaria.

During the summer of 1905 I paid a visit to the Biological Station at Heligoland with the object of obtaining materials for a renewed study of the development of Phoronis. I was at the same time enabled to examine living larvæ of Echinoderms and a few Tornaria.

Preliminary results upon certain points in the structure of these larvæ were indicated in 1898, but until 1905 I was unable to obtain sufficient material for definite conclusions.

One of the most detailed descriptions of Tornaria at various stages is that of Morgan,[1] who has also followed its development into the adult, in the case of at least two species; I have had occasion to make constant reference to his results.

It may be recalled that the general surface of Tornaria is even more sharply divided into two areas than that of Echinoderm pelagic larvæ, by the sinuous course of a ciliated band usually known as the "circum-oral band" (Morgan). The area lying within this band is known as the circum-oral area in contradistinction to the remainder or the extra-oral area. The circum-oral area is very thin-walled, and is, at least in older stages of Tornaria, more or less sunk inwards below the level of the thicker-walled extra-oral area. It is bounded

[1] Morgan, T. H., "Journal of Morphology," vols. v and ix.

everywhere by the circum-oral band carrying long cilia, but is not itself ciliated except in one particular part (see below).

The general arrangement of the area is a combination of bilateral (or planar) and axial symmetry. The bilateral symmetry is perfect. The band runs away from the region of the mouth in a complicated loop which is identical on either side of the body. There can usually be distinguished an antero-ventral, an antero-dorsal, and a postero-dorsal band. The original part of the band leading away from the mouth forms a postero-ventral fold joining with its fellow across the mouth.

A view of the larva from the anterior end shows that the four anterior folds are arranged symmetrically round the central axis and tend towards an axial symmetry, which becomes more pronounced in the most complex larvæ (cf. Morgan's "Bimini larva").

The phyletic history of this band is clearly a gradual extension of a bilateral loop, conforming by degrees to the axial symmetry of the larva. It does not appear, in the North Sea larva, to form loops or tentacles in its course. The posterior band remains as a simple circular band in all known Tornariæ, and traverses the posterior part of the extra-oral area.

An examination of the living larva shows that this extra-oral band, like the peri-anal band of Actinotrocha, is purely motor in function. Only when its cilia are in action does the larva move with any activity. The cilia are curved, and all strike the water backwards, with a resultant effect along the antero-posterior axis of the larva. Its purely motor function explains the limitations of the band at all stages to a simple circle symmetrical with the long axis.

On the other hand, the circum-oral band does not appear to any great extent to subserve locomotion. On occasions when the extra-oral band is not in motion the circum-oral band may be very active with but little or no effect on the general movements of the larva. The main function of the circum-oral band appears to be that of food-

ingestion, i. e. the collection of food-particles towards the mouth. The complication of the band and its attendant increase in size of the larva, is probably traceable to a progress towards more efficient ingestion of food by an increase of ingestive surface. The same distinction into motor and trophic bands can be made out, as has been indicated, in echinoderm larvæ.[1] The action of the circum-oral band in collection and transmission of food is complex, and several points remain to be determined, but in the immediate neighbourhood of the mouth the food-particles can be watched with ease as they are waved towards the mouth opening and pass rapidly into the alimentary canal.

The mouth opens into the circum-oral area at a point on the median ventral line. A modified portion of the area in the immediate neighbourhood of the mouth may be distinguished as the vestibule, or buccal cavity. Fig. 11 is a view of the living larva, showing the anterior and posterior parts of the circum-oral band bending forwards in a loop, at the apex of which the mouth is seen.

The limits of the vestibule are clearly distinguishable. It forms a wide chamber, open laterally to the remainder of the circum-oral area. Its floor, or ventral wall, has a patch of cilia forming a more or less complete connection between the circum-oral band and the floor of the pharynx. This patch (or buccal pad) (Fig. 12, *b.p.*) has already been noticed in American Tornariae by Morgan (loc. cit.) Apparently it tends to disappear or to become diminished in size in the fully-grown larvæ. It is probably the action of the buccal cilia which is chiefly instrumental in bringing the food particles into the mouth. In the Bahama larva of Morgan the mouth does not, in the later stages, open directly to the exterior, but into a long ectodermal tube, up which the ciliated bands can be traced. No such tube appears to be developed in the Heligoland larva. The first portion of the alimentary canal is usually known as the œsophagus. It seems desirable to distinguish this part as the "pharynx,"

[1] 'Quart. Journ. Micr. Sci.,' vol. xliii.

because it actually becomes the pharynx of the adult, and it appears to have a function and a relationship with the remainder of the alimentary canal, which is usually more identified with a pharynx than an œsophagus. Morgan, in describing early stages of the New England Tornaria, remarks that it first consists of a simple tube, quadrilateral in outline, with long cilia throughout its length.

"The cilia on the dorsal wall of the œsophagus do not continue into the stomach, whilst those over the ventral side continue, after a slight interruption at the groove, as a narrow band down the anterior wall of the stomach; the cilia become smaller as the band runs down the wall, and cease about the middle of the stomach" (p. 418).[1]

In the advanced larvæ which I have examined the arrangement is rather more complex. The pharynx (œsophagus) is a conical tube with its narrow end towards the stomach. Its cross section is never quadrilateral. The outline of the mouth itself is seen in Fig. 11 as it appears in the living animal; an enlargement of the same view is shown in Fig. 16. It is about twice as broad as it is long (antero-posteriorly). The two lateral corners are pulled out into loops, whilst the central part is arched dorsally and ventrally. The two lateral grooves are not on the same level as the central portion, but run backwards down the lateral walls of the pharynx. The dorsal arch of the mouth is continued backwards throughout the length of the pharynx, and is lined with cilia (*d.e.*) down to the commencement of the stomach. The ventral portion (*v.c.*) is strongly ciliated throughout its course. Anteriorly the cilia are practically continuous with those of the buccal pad, and posteriorly they pass into the stomach. Here they expand into another large pad, the gastric pad (*g.p.*), which appears to be a specialised portion of the more diffusely distributed stomach-cilia described by Morgan. This gastric pad is a conspicuous feature of the living larva (cf. Figs. 11 and 15).

The lateral grooves are continued down the walls of the

[1] Morgan, T. H., 'Journal of Morphology,' vol. ix.

pharynx as a pair of long channels (*pl.*), which are distinguished by an absence of cilia.

All these structures may now be followed in sections.

Figs. 1 to 4 are a series of coronal or horizontal sections through Tornaria. The circum-oral and extra-oral areas are easily traceable through the depression of the former into the body of the larva and its very thin limiting walls.

Fig. 1 is ventral to the mouth, and shows the circum-oral area crossing the ventral surface of the larva as a V, with the apex directed forwards. The anterior part of the circum-oral band (*c.o.a.*) is cut in four places, and the extra-oral band twice (*ex.*).

In the middle line the circum-oral area becomes the vestibule, and its roof, or dorsal wall, is seen to be slightly thickened. The posterior part of circum-oral band is cut in its length across the floor of the vestibule, and in two other places (*c.o.p.*) the dorsal wall of the vestibule is thick, and often carries small cilia.

In Fig. 2 the edge of the mouth itself is cut. The thick dorsal and ventral walls of the pharynx are seen, and the left lateral groove (*l.g.*) is continued outwards into the circum-oral area. The right side is cut further back, and the lateral groove is no longer in communication with the circum-oral area.

In Fig. 3 the pharynx is cut nearly in cross-section, and in Fig. 4 it is seen to join the stomach.

Figs. 5 to 9 are a series of sections of the pharynx more highly magnified. Fig. 5 shows the lateral groove open on the left side and closed on the right. This and the following sections show the histological characters of the pharynx. It consists throughout of a single layer of cubical elongated cells. In the dorsal and ventral parts these cells carry cilia, but those surrounding the lateral grooves have none. These cilia are far shorter than in the living larva, and have either contracted considerably or have been broken off. The lateral grooves show a number of clear vesicles or vacuoles arranged rather indefinitely, and varying in number accord-

ing to the age of the larva. These vacuolations are identical with those described in the pharynx of Actinotrocha, and are apparently the first stages in the production of chordoid tissue found in the adult pharynx of Balanoglossus and Cephalodiscus. Morgan gives a figure of the œsophagus, in cross-section, of the youngest Bahama larva. It shows the same condition as regards the arrangement of the cilia, i. e. dorsal and ventral ciliated areas and an entire absence of cilia from the lateral regions.

No allusion to this structure is made in the text, and the later stage seems according to the figures to have a different disposition of the cilia. Morgan makes no allusion to the vacuolation. Larval stages can be obtained which show all the main features here outlined without any trace of the vacuolations, which appear to arise generally at a late stage.

The pharynx of Tornaria (at this stage) therefore consists of a long funnel-shaped tube, narrowing inwards and depressed dorso-ventrally at the outer end. Its dorsal and ventral surfaces are ciliated throughout, and in the living condition tend to become depressed, especially the ventral wall, into grooves. Down each side there runs a lateral groove, the cells of which are non-ciliated and tend to become vacuolated. Each of these lateral grooves passes out at the corner of the mouth into the vestibule. This condition reminds one irresistibly of the pharynx of Cephalodiscus, as will be explained later. The two lateral grooves correspond with the pleurochords in Cephalodiscus, with the difference that they do not terminate in closed tubes or pharyngeal clefts, but in oral grooves. Down the centre of this pharynx food-particles can be seen to pass in a current which sets steadily towards its inner termination. I have failed to trace the return current of water chiefly because it is devoid of food particles, and is very difficult, if not impossible, to see. The particles gather in a batch at the end of the pharynx, the entrance of which to the stomach is closed. It is difficult to understand how the water can find

its way back except along the lateral grooves and out at the sides of the mouth.

Intermittently the end of the pharynx opens, and the particles are shot into the capacious stomach in a scattered heap.

The stomach (*st.*) is a large globe with rather thin walls. Just ventral to the pharynx is the peculiar thickened gastric pad or disc (*g.p.*), which is strongly ciliated. I have not yet determined the exact function of this pad, but it is apparently concerned with the introduction of particles into the stomach, and may possibly assist in the production of the food currents. At the posterior end of the stomach a central aperture leads into the intestine. Usually this is closed, and from its closed edges there projects a long brush of cilia. These are usually in very active motion. Their resultant effect is to drive a column of water with its particles up the centre of the stomach towards the anterior end; here the current spreads down the walls of the stomach on all sides till it reaches the posterior end, when it again converges inwards to form the centre column (Fig. 14).

These movements appear to be very characteristic and show little variation. They continue without intermission for any period from half an hour to two hours, or more.

Running the length of the stomach, in a ventro-lateral position, is a pair of thickened areas (Fig. 4) which appear to be identical in structure and function with the "digestive areas" which I have described in Actinotrocha.[1] Similar areas are indicated in a cross-section of Tornaria by Morgan, but no reference to them is made in the text. This forms one of many smaller anatomical resemblances which confront at every turn any investigator of the two types.

Sooner or later the currents slow down, and the food-particles collect at the posterior end. The posterior wall of the stomach and the anterior wall of the intestine are pressed close together to form a sort of diaphragm which is seen to open from the centre like an iris till a large and gaping com-

[1] 'Quart. Journ. Micr. Sci.,' vol. xl.

munication is established between the two chambers, and the food particles are ejected in a shower into the intestine. The diaphragm then closes up in the reverse manner. The whole operation is performed methodically, and takes about fifteen seconds to open, a pause, and, perhaps, about the same time to close. There are no muscles, and apparently the protoplasmic walls of the stomach and intestine act together in an ordinary contraction.

On the intestinal side of the aperture there is a group of cilia of a smaller size than those in the stomach, projecting backwards into the cavity of the former.

Apparently when the diaphragm opens the walls are contracted up into a belt which depends into the intestine (Fig. 13), and in this belt region the walls are perceptibly thickened. From its upper rim the stomach cilia depend into the intestine, and, as a rule, when the aperture closes these cilia are caught in this position, but they can be seen to be gradually withdrawn, doubled upon themselves, until they are once more in their normal position. The movement reminds one of the action of a swan bringing its head and neck above water (cf. five stages of Fig. 13).

The intestine (*int.*) is a large cone, with the anus at its apex. It does not appear to be generally ciliated and its walls do not differ essentially from those of the stomach. In its cavity the food-particles are rapidly rotated (Fig. 14). Usually the column of water moves up the centre and down the outer walls so that the particles pass towards the centre at the anal end. I have seen this current reversed. In either case the current appears to be caused by the ring of cilia around the pylorus.

Ciliary motion of a lesser nature also appears to take place around the inside of the anus, and a very ring of cilia can be often made out in this region.

The anal aperture (*a.*) opens in much the same manner as the pylorus by protoplasmic contraction of the surrounding walls, which draw away from each other till a fairly wide aperture is made and the remains of the food are ejected

forcibly to the exterior. The action of the pylorus and that of the anus could not be performed more methodically and efficiently if they had been provided with nerves and sphincter muscles.

Summarily, we may state that—

Ingestion is effected by ciliary currents.

The extra-stomial ingestion is effected by the circum-oral band, and by the ciliated walls of the vestibule.

Intra-stomial ingestion is effected by the cilia lining the pharynx.

The water of the ciliary currents is probably returned along the lateral grooves of the pharynx, and then by the corners of the mouth to the exterior.

Digestion is intra-cellular in the stomach (digestive areas), but may also be inter-cellular—in the stomach, and in the intestine also.

Currents in the stomach and intestine are ciliary.

The pylorus and anus are worked by the rhythmic contractility of the surrounding walls.

Enteropneusta and Pterobranchia.

In the last communication of this series[1] some points in the structure of Cephalodiscus dodecalophus were dealt with, mainly concerning the central complex of pericardial sac, glomerulus, heart, and other parts of the blood, vascular, and nervous systems. Many of these points have since been confirmed by Schepotieff and Harmer,[2] and the general result is to add considerably to the anatomical resemblances of this type to the Enteropneusta. There still, however, remains the important difference due to an absence of metameric segmentation in the pharyngeal clefts and the gonads of the Pterobranchia.

[1] 'Quart. Journ. Micr. Sci.,' vol. xlvi.

[2] 'Siboga-Expeditie,' Monographie xxvi.

Lang was, I believe, the first to suggest an explanation of this difference as being accountable to the sedentary habits of Cephalodiscus, which has presumably resulted in a loss of all but one pair of the pharyngeal clefts and gonads.

The same view has been held more recently by Willey,[1] to whom the assumption is apparently necessary for his theory of the primary origin of pharyngeal clefts, as a cleft, according to him, can apparently only arise between two contiguous gonads.

According to Willey pharyngeal clefts have arisen primarily as branchial clefts, or clefts connected with respiration of the gonads, and they have only secondarily lost this function and become connected with the removal of water in relation to alimentation.

In a paper read in 1898[2] I suggested a theory of the origin of pharyngeal clefts which attaches no primary importance to the contiguity of the clefts and the gonads in Enteropneusta. It was assumed that, upon the first evolution of metameric representation in the trunk of Enteropneusta, the gonads and clefts became repeated together in regular order from a primary Pterobranchian arrangement of one pair of each,—a view which is entirely opposed to, and incompatible with, Willey's suggestions. There appear to be several further difficulties in the way of accepting Willey's hypothesis. Firstly, there is no evidence of any kind that the Pterobranchia or Phoronis ever had more than one pair of clefts or gonads, the probability of which has been urged by him. Secondly, there is doubt as to whether the clefts in Enteropneusta really subserve respiration of the gonads in a direct manner. The necessity for such an arrangement does not appear obvious when we recall the existence of a large number of Annelid animals with large gonads embedded deep in the tissues, but with no special adaptation for direct aeration of these organs. Thirdly, the facts here described show that the larval Enteropneusta

[1] 'Quart. Journ. Micr. Sci.,' "Enteropneusta," Willey's Zoological Results.

[2] 'Report of the British Association" (Bristol), 1898.

have a pharynx in which there are simple paired pleurochords, terminating in lateral oral grooves.

Morgan[1] has shown that this portion of the pharynx of Tornaria, here identified as the pleurochords, grow outwards in the course of development into serial pouches, which eventually reach the ectoderm and form the pharyngeal clefts, the pleurochords themselves remaining as the two halves of the dorsal part of the adult pharynx, into which the clefts open in the adult, whilst the ventral part expands to form the ventral portion of the adult pharynx. As soon as the first pair of pharyngeal clefts arises in Tornaria its pharynx is then, in its essential features, comparable with that of the Pterobranchia, passing on to that of the adult Enteropneustan by the production of further pharyngeal clefts along the course of the pleurochords. The conclusion appears to be fairly evident that the Enteropneustan pharynx must be regarded as a further elaboration of the type found in Pterobranchia, or, in other words, that the Enteropneusta are essentially Diplochorda, though this is disguised in the adult by a general vacuolation of most of the pharynx and the production of a large number of pharyngeal clefts. Harmer[2] has, on more than one occasion, recorded an emphatic objection to the adoption of this term, but such results as the above seem to show that it expresses a fundamental character of these animals. Even if the name be not adopted in classification, the underlying character of the presence of true lateral pharyngeal pleurochords cannot be ignored. Harmer goes no further than comparing these organs in Pterobranchia with "the dorsal part of the pharynx of Balanoglossus," a comparison which agrees precisely, so far as it goes, with the view here expressed.

Recent work upon Rhabdopleura[3] (Schepotieff) has shown that this type also possesses two pharyngeal pleuro-

[1] 'Journal of Morphology,' vol. ix.

[2] 'Zool. Anzeiger,' xx, 1897, et loc. cit.

[3] Bergens Museums, Aarbog. 1904; 'Zoolog. Anz.,' xxviii, 1905

chords which terminate in a pair of well-marked oral grooves, or that the pharynx of Rhabdopleura is comparable with that of other Diplochorda. Fresh anatomical resemblances between Rhabdopleura and Pterobranchia have recently been brought out by Harmer[1] and Ridewood.[2] These need not be recapitulated here, but I believe that when their full significance is taken into consideration they will necessarily have to be expressed in classification. At present I regard the Diplochorda as a natural group comprised of at least three natural divisions, namely, Enteropneusta, Pterobranchia, and Rhabdopleura, and possibly the Phoronidea.

The name appears to me to emphasise a fundamental character, which now is shown to be characteristic of the whole group.

The monograph of Harmer to which repeated reference has been made contains, in addition to a number of new observations on the anatomy of Pterobranchia, a discussion of the inter-relationship of types I have attempted to enlign under the title Diplochorda. In his allusions to my work on Actinotrocha the impression is given that the subsequent workers on similar material have concurred in a general disagreement with my results.

Whilst fully cognisant of certain errors of observation and of interpretation in the early work alluded to, I feel that progress would be facilitated by pointing out that the subsequent authors have differed in their results inter se, quite as much as, if not more than, with mine. Until a complete series of developmental stages, with ample material, is worked out, this state of affairs is likely to continue.

In the meantime I wish to guard against a false impression till the whole subject can be dealt with later in greater detail.

[1] 'Siboga Expeditionie,' Mono. xxvi bis.

[2] 'Natl. Antarctic Expedition,' vol. II, Pterobranchia.

EXPLANATION OF PLATE 29,

Illustrating Dr. A. T. Masterman's paper "On the Diplochorda. Part V. Certain Points in the Structure of Tornaria."

List of Abbreviations.

a. Anus. *a.b.* Adoral band. *b.p.* Buccal pad. *cœ.* Cœlom. *c.o.a.* Anterior part of circum-oral band. *c.o.p.* Posterior part of circum-oral band. *d.c.* Dorsal cilia of pharynx. *ex.* Extra-oral band. *g.p.* Gastric pad. *hy.* Hydrocœle. *int.* Intestine. *m.* Mouth. *l.g.* Lateral groove. *œs.* Œsophagus. *p.a.* Peri-anal band. *p.c.* Pre-oral cœlom. *ph.* Pharynx. *ph.d.* Dorsal chordoid wall of pharynx. *pl.* Pleurochord. *p.v.* Proboscis vesicle. *st.* Stomach. *v.* Vestibule. *v.c.* Ventral cilia of pharynx.

Figs. 1–4.—Selected sections from a frontal series through a Tornaria larva. They are the 4th, 7th, 11th, and 16th respectively of the series.

Figs. 5–9.—The pharyngeal region of the same series, seen with higher magnification.

Fig. 5 is part of Fig. 2, the 7th section of the series.

Fig. 6 is part of Fig. 3, the 11th section of the series.

Fig. 7 is from the 18th section of the series.

Fig. 8 is from the 15th section of the series.

Fig. 9 is from the 22nd section of the series.

Fig. 10.—A dorsal view of the entire Tornaria.

Fig. 11.—Latero-ventral view (left side) of a slightly later stage of Tornaria; more highly magnified.

Fig. 12.—Lateral view of the pharynx of Tornaria.

Fig. 13.—A series of optical sections of the opening between stomach and intestine of Tornaria, drawn to show consecutive stages in the closure of the opening.

Fig. 14.—Optical section of the stomach and intestine of Tornaria to show alimentary currents.

Fig. 15.—Lateral view of pharynx and stomach of early Tornaria.

Fig. 16.—Frontal view of the mouth and pharynx of Tornaria.

The Structure, Development, and Bionomics of the House-fly, Musca domestica, Linn.

Part II.—The Breeding Habits, Development, and the Anatomy of the Larva.

By

C. Gordon Hewitt, M.Sc.,
Lecturer in Economic Zoology, University of Manchester.

With Plates 30—33.

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I. Introduction.

In the present paper, which is the second of the series of three, the breeding habits and development of M. domestica and the anatomy of the mature larva will be described. Its publication has been delayed owing to the fact that I wished to make the observations on the breeding habits and life-history as complete as possible. With the recent appearance of two short papers by Newstead (1907) and Griffith (1908), many of whose observations, to which I shall refer later, are confirmatory of my own results, we now have a more complete account of the breeding habits of this insect.

The anatomy of the larva has been described in a similar manner to that of the fly (1907). I have refrained in this paper from giving a detailed account of the embryology and the development of the imaginal discs, as these are separate and specialised studies, and would have resulted in too great a digression from the plan originally adopted.

The methods used were the same as those previously employed. The anatomical structures were studied with the aid of the Zeiss binocular dissecting microscope, and the drawings were made from the dissections. The latter were confirmed by means of serial sections. Too much stress cannot be laid on the importance of employing both these methods where possible, as it frequently happens that mistakes are made in investigating by one method only, which would be unrectified were not the other employed in confirmation.

I wish to thank the Council of the Manchester University for providing me with a suitable experimental greenhouse and apparatus for the experimental portion of this investigation; the absence of such facilities would have been a severe handicap. The outdoor observations on the breeding habits have been made during the last few years in Manchester and the surrounding district.

The third paper, which will conclude this study of M. domestica, will deal with the bionomics of the fly, its para-

sites and its relation to man, and certain of its allies which frequent houses will be considered.

II. Breeding Habits of M. domestica.

The development of M. domestica was first described by Carl de Geer (1776); but, although he stated that it developed in warm and humid dung, he did not give the time occupied by the different developmental stages. He refers to the enormous quantities of flies occurring from July to August. His statement concerning their development is especially interesting, as he appears to be the first investigator who called attention to what I consider to be one of the most important factors in the development of the fly, namely, the process of fermentation occurring in the substance in which development is taking place. He says (p. 76), "Les larves de cette espèce vivent donc dans le fumier, mais uniquement dans celui qui est bien chaud et humide, ou pour mieux dire *qui se trouve en parfaite fermentation*" (the italics are mine). Since the completion of my own investigations on the development, all of which indicated the importance of this factor fermentation, Newstead (l. c.) has come to the same conclusion. The work of Keller (1790), to which reference was made in the first part of this memoir, contains many interesting and careful observations on the breeding habits of the "Stubenfliege." He found that the eggs hatched from twelve to twenty-four hours after deposition. He reared the larvæ in decaying grain where, no doubt, fermentation was taking place; also in small portions of meat, slices of melon, and in old broth. His observations are extremely interesting, and, excluding mistakes which were due to the lack of modern apparatus, his account is still a valuable contribution to our knowledge of the subject. Bouche (1834) describes the larvæ as living in horse-manure and fowl-dung, especially when warm. He does not give the time occupied by the earlier developmental stages, but states that the pupal stage lasts from 8—14 days. Packard (1874)

was the next to study the development and working in the United States of America at Salem, Mass., he found that the larvæ emerge from the eggs twenty-four hours after deposition; the times taken by the three larval stages—for he found that there were two larval ecdyses—were: first, about twenty-four hours; the second stage, he thought, was from twenty-four to thirty-six hours; and the third was probably three or four days; the entire larval life being from five to seven days. The pupal stage was from five to seven days, so that in August, when the experiments were carried on, the time from hatching to the exclusion of the imago was ten to fourteen days. Taschenberg (1880) incorporates the work of Keller and Bouche, and does not appear to add anything of importance to the facts already mentioned. He states that the female flies deposit their eggs in damp and rotting food-stuffs, bad meat, broth, slices of melon, dead animals, cesspools, and manure-heaps. He further says that they have also been observed laying their eggs in spittoons and open snuff-boxes. With reference to the last statement, I find that the larvæ will feed on expectorated matter mixed with a solid substance, such as earth, if they are kept warm, though they cannot feed on salivary sections merely; but, although flies might improvidently deposit their eggs in an open snuff-box, the larvæ would soon perish on hatching on account of the dry conditions.

Howard (1896—1906) first studied the breeding habits of the fly in 1895 in Washington, U.S.A., and he described them in 1896, and more fully subsequently. He found that they could be rarely induced to lay their eggs in anything but horse-manure and cow-dung, and that they preferred the former. The periods of development he found were as follows:—from the deposition of the egg to the hatching of the larva about eight hours; the first larval stage one day; second larval stage one day; third larval stage—that is, from the second ecdysis to pupation—three days, and the flies emerged five days after the pupation of the larvæ, thus making the whole period of development about ten days. The same

author in a valuable study of the insect fauna of human excrement (1900), describes experiments in which he was successful in rearing M. domestica from human excrement both in the form of loose fæces and in latrines. Newstead (l. c.), in addition to confirming some of my observations, also found the larvæ in spent hops, dirty beddings from rabbits and guinea-pigs, bedding from piggeries, and in the rotten flock beds and straw mattresses which, I suppose, were either in, or from, ashpits, and fouled with excremental products, although it is not stated. He appears to have overlooked some of the work of previous investigators.

My studies of the breeding habits of M. domestica were initiated in 1905, and were continued in 1906, when a short account of some of the results was published (1906). The shortest time which I then obtained for the development of any batch of larvæ was twenty days, although, taking the shortest period obtained for each developmental stage in the series of experiments, the development could have been completed in fifteen days under suitable conditions. In the summer of 1907 I continued my experiments on a much larger scale and under better circumstances, and the following are the results of my experiments and outdoor observations:

The larvæ have been successfully reared in horse-manure, cow-dung, fowl-dung, human excrement, both as isolated fæces and in ashes containing or contaminated with excrement, obtained from ashpits attached to privy middens, and such as is sometimes tipped on to public tips. I found that horse-manure is preferred by the female flies for oviposition to all other substances, and that it is in this that the great majority of larvæ are reared in nature; manure-heaps in stable yards sometimes swarm with the larvæ of M. domestica. It was also found that the larvæ will feed on paper and textile fabrics, such as woollen, cotton garments, and sacking which are fouled with excremental products if they are kept moist and at a suitable temperature. They were also reared on decaying vegetables thrown away as kitchen refuse, and on such fruits as bananas, apricots, cherries, plums, and peaches,

which were mixed, when in a rotting condition, with earth to make a more solid mass. Although they can be reared in such food-stuffs as bread soaked in milk and boiled egg, when these are kept at a temperature of about 25° C., I was unable to rear them to maturity in cheese, although they fed on the substances for a few days and then gradually died, my failure may have been due to the nature of the cheese which was used, only one kind being tried. In addition to rearing the larvæ on isolated human fæces, such as are frequently found in insanitary court-yards and similar places, they were found in privy middens, and also on a public tip among the warm ashes and clinker where the contents of some privy middens had also evidently been emptied; I bred the flies out from this material.

III. Factors of Development.

The rate of development depends primarily on the temperature of the substance on which the larvæ are feeding. This was shown in my experiments in which the larvæ were reared in horse-manure kept in a moist condition in an incubator at a constant temperature of 35° C. At this temperature the development is completed in eight to nine days. I found that a higher temperature of 40° C. was too great for the larvæ as they were simply cooked and perished at such a temperature. This has been confirmed by Griffith (l. c.), who found that the life-history was completed in the same time on incubating at a temperature of about 22°—23° C. I do not think that a shorter time than this for the development—that is, from the deposition of the egg to the emergence of the perfect insect from the pupæ—will ever occur in this country, as we rarely enjoy prolonged spells of hot weather which would bring about such conditions as regards temperature. It is interesting to note that Smith (1907) gives the time of development in horse-manure in India under natural conditions as eight days; he also bred M. domestica from an artificial latrine containing human excreta mixed with earth, which confirms

English observations for India. In England, during a period of extremely hot weather, flies might develop in about nine days, but such a rate of development would not usually occur, nevertheless, as I shall show in the concluding part of this memoir, such a contingency must be guarded against. Larvæ reared in the open air in horse-manure which had an average, but not a constant, daily temperature of 22·5° C., occupied fourteen to twenty days in their development according to the air temperature.

The effect of the character of the food on which the rate of development also depends is well shown by a comparison of the times of the developmental periods in two of the experiments where the average daily temperature was practically the same, namely, 19·3° C. and 20·5° C. In the former experiment, in which human fæces were used, the development was completed in twenty days, and in the latter, in which bananas were used, the development occupied twenty-seven days; the time was rather lengthened in both cases by the fact that the larval food was rather dry, but equally dry in both experiments as they were kept together; had more moisture been present the times would probably have been correspondingly shortened.

It was experimentally proved that when larvæ were reared, in batches on the same kind of food material with conditions, as regards temperature the same, the developmental period was longer for those larvæ which were subject to dry conditions than for those subject to moist conditions. In an experiment at an average temperature of 22° C. larvæ reared on horse-manure which was kept in a rather dry condition took thirty days to complete the development, and another batch at the same temperature, but on horse manure which was kept moist, the development was completed in thirteen days. Under similar conditions, with regard to temperature, the rate of development is directly proportional to the condition of the food as regards moisture. Dry conditions not only retarded development in some of my experiments to five and six weeks, but also tended to produce flies of sub-

normal size. Moisture is necessary for the development, and if the food becomes too dry the result is fatal, as the larvæ perish.

A fourth and a most important factor affecting development and one intimately connected with the previous factors—temperature, character of food, and moisture—is that of fermentation, to which reference has already been made. This process appears to take place in the substances on which the larvæ best subsist. Whether the suitability of the food is determined by the nature of its fermentation is a point which I was unable to determine, but which I am inclined to believe. I feel certain, however, that the calorific property of fermentation is the most important part of this process on account of its direct relation to the time of development; the endogenous heat of excremental products and decaying substances acting either in addition to, or independently of, the temperature of the surrounding air is of great advantage in accelerating the rate of development.

The Rate of Development.—This was never found to be less than eight days, and was more usually twelve to twenty days owing to the fact that a continuously high air temperature was not maintained for any sufficient length of time; with such a continuous period of hot weather the development would take about ten to twelve days, and in very great heat might be completed in a day or two less as the internal temperature of the breeding places, such as manure-heaps is usually higher than the temperature of the air. It must be remembered, however, that except by incubation it is difficult to experimentally imitate such natural conditions as occur in a manure-heap or privy midden, where, owing to a larger amount of material, a higher constant temperature is maintained. All experimental results except those of incubation tend to give a long rather than a short rate of development. In many cases where the average temperature was 20° C., but the food material rather dry, the developmental period was about three weeks, and where the temperature was low and the food became dry it extended to

as much as six weeks, the greater time being spent in the pupal state which was sometimes of three or four weeks duration.

In no case was I able to keep the pupæ through the winter as I have been able to keep the pupæ of Stomoxys calcitrans and other forms.

My experiments and observations point to the fact that in the presence of suitable larval food, such as excremental matter or decaying and fermenting food materials in a moist and warm condition, the female flies would lay their eggs and the larvæ would develop if the temperature of the air were sufficiently high for the prolonged activity of the flies. In winter this last condition is not naturally satisfied, but under such conditions as are found, for example, in warm restaurants and kitchens, stables and cowsheds, female flies may be often found during the winter. On dissecting such flies I have found mature ova in the ovaries, and living spermatozoa in the spermathecæ, which facts support this view. Griffith (l. c.) has succeeded in rearing batches of eggs in November, December, and early January under artificial conditions, which further proves their ability, given the necessary conditions as regards temperature, to breed during the winter months. In this country M. domestica breeds, as a rule, from June to October, and the greatest egg-laying activity prevails in August and September. As I have already contended, and as Griffith has shown, they may breed at other times if the necessary conditions are present; I have obtained eggs from flies caught in restaurants in December; Keller also mentions the fact that he obtained eggs in January. These facts may account for the rapid appearance of flies in the early summer. It is not unlikely that the flies which survive the winter months, which many spend in a dormant condition if they are not fortunate enough to remain active in a warm restaurant or stable, lay their eggs, almost immediately on renewing their activity, in such places as manure-heaps which are kept, as is often the case in towns, under cover, and which are consequently warmer externally than those in the open. In this way a large number of flies

would be reared and ready to assume their customary activity under the benign influence of the sunny days of June.

I have made many experiments with a view to finding out the rapidity with which house-flies breed. Anyone who has endeavoured to keep flies alive in an enclosed space will appreciate the difficulty of the task, those who have not done so would hardly realise it. Fewer insects seem less tenacious of life when enclosed even in a comparatively large enclosure of six or nine cubic feet. It is a remarkable fact, as one would imagine a priori that these insects, flying about everywhere as they do, could be easily kept in a roomy cage if given the necessary food and water. This, however, has not been the case in my experience ; the longest period which I have been able to keep them in captivity in summer is seven weeks. I am pleased to find that Griffith has succeeded in keeping a male fly sixteen weeks, and has obtained four batches of eggs from females in captivity. In one of my experiments I was successful in obtaining flies of the second generation bred in captivity. I found that the flies became sexually mature in ten to fourteen days after their emergence from the pupal state and, four days after copulation, they began to deposit their eggs, that is, from the fourteenth day onwards from the time of their emergence.

From these results it may be seen that in very hot weather the progeny of a fly may be laying eggs in about three weeks after the eggs from which they were hatched had been deposited. As a single fly lays from 120—150 eggs at one time and may deposit five or six batches of eggs during its life, it is not difficult to account for the enormous swarms of flies that occur in certain localities during the hot summer months, and algebraical calculations are not required to more vividly impress the fact.

IV. Development.

As I have already stated, M. domestica may become sexually mature in about ten to fourteen days after emer-

gence from the pupal case, and at this time they may be seen copulating.

1. The copulation of M. domestica appears to have been first described by Reamur (1738). It has been carefully described recently by Berlese (1902), whose observations my own confirm. The male may perform a few tentative operations before copulation takes place, and these have been mistaken for the actual act. The male alights on the back of the female by what appears to be a carefully calculated leap from a short distance, and this act seems to indicate a faculty on the part of the fly of being able to judge distance. It then caresses the head of the female, bending down at the same time the apical portion of the abdomen. The male fly is, however, peculiarly passive during the operation, its influence apparently being only tactual, it is only when the female exerts her ovipositor and inserts it into the genital atrium of the male that copulation can successfully take place. When the ovipositor has been inserted into the genital atrium of the male, the accessory copulatory vesicles of the female become turgid and retain the terminal segment in this position, in which the female genital aperture is situated opposite to the male genital aperture at the end of the penis, the latter depending from the roof of the genital atrium. (This will be better understood by reference to the figures of these parts in Part I of this Memoir). The attachment of the penis to the female genital aperture is made still firmer by the dorsal sclerites of the eighth segment of the female and the ventral sclerites of the seventh segment, the so-called secondary forceps of the male acting respectively above and below the penis. The fifth ventral segment, or primary forceps of the male, assist the accessory copulatory vesicles of the female in preventing the withdrawal of the ovipositor before the spermatozoa have been injected into the female genital aperture, by which way they enter the spermathecæ. The whole act may be over in a few moments or they may remain in coitu for several minutes.

The eggs are laid a few days after copulation; I found

that oviposition may take place as early as the fourth day; Taschenberg (t. c.) states that the female lays on the eighth day after copulation. When about to deposit its eggs the fly alights on the substance which it selects as a suitable nidus and, if possible, crawls down a crevice out of sight. There it lays its eggs in clumps; they are usually placed vertically on their posterior ends and closely packed together. During a single day, if undisturbed, a fly may lay the whole batch of eggs which are mature in the ovaries and which may number, I find from actual count, from 120—150.

2. The Egg.—The egg of M. domestica (Pl. 30, fig. 1) measures 1 mm. in length, sometimes slightly less. It is cylindrically oval; one end, the posterior, is broader than the other, towards which end the egg tapers off slightly. The outer covering or chorion is pearly white in colour, the polished surface being very finely sculptured with minute hexagonal markings. Along the dorsal side of the egg are two distinct curved rib-like thickenings having their concave faces opposite. In the hatching of the eggs which I have observed, the process was as follows:—A minute split appeared at the anterior end of the dorsal side to the outside of one of the ribs; this split was continued posteriorly (fig. 2), and the larva crawled out, the walls of the chorion collapsing after its emergence. The time of hatching varies according to the temperature. With a temperature of 25°C.—35°C. the larvæ hatch out from eight to twelve hours after the deposition of the eggs; at a temperature of 15°C.—20°C. it takes about twenty-four hours, and if kept as low as 10°C., two or three days elapse before the larvæ emerge.

3. The Larva.—First larval stage or first instar. —The newly-hatched larva (fig. 8), measures 2 mm. in length. It contains the same number of segments as the mature larva and at the anterior end of the ventral surface of each of the posterior eight segments there is a spiny area (*sp.*). The posterior end is obliquely truncate, and bears centrally the only openings of the two longitudinal tracheal trunks, each trunk opening to the exterior by a pair of small oblique slit-

like apertures situated on a small prominence (*p.sp.*). There are no anterior spiracular processes in the first larval stage. The oval lobes are relatively large and on the internal ventral surface of each there is a small T-shaped sclerite (fig. 13, *t.s.*). These sclerites lie lateral to the falciform mandibular sclerite (*m.s.*). The cephalopharyngeal skeleton of the first larval instar is slender and, in addition to the sclerites already mentioned, consists of a pair of lateral pharyngeal sclerites or plates (*l.p.*) deeply incised posteriorly, forming well pronounced dorsal and ventral processes. The lateral plates are connected antero-dorsally by a curved dorsal sclerite (*d.p.s.*). The anterior edges of the lateral plates are produced ventrally into a pair of slender processes (*h.s.*), the anterior portions of these processes, which represent the hypostomal sclerite, are involute and articulate with the mandibular sclerite. The alimentary canal of the first larval instar is relatively shorter than that of the adult, and consequently it is not so convoluted; the salivary glands are proportionately large.

The first larval instar may undergo ecdysis as early as twenty hours after hatching, but it is usually from twenty-four to thirty-six hours that the ecdysis takes place: under unfavourable conditions with regard to the factors governing the development, the first larval instar sometimes lasted three or four days. Ecdysis begins anteriorly, and the larva not only loses its skin but also the cephalopharyngeal sclerites which are attached to the stomodæal portion of the ecdysed chitinous integument; the chitinous lining of the proctodæal portion of the alimentary tract is also shed.

The second larval stage or second instar. This stage is provided with a pair of anterior fan-shaped spiracular processes similar to those of the mature larva. The posterior spiracular orifices are shown in fig. 12. They are slit-like apertures rather similar to those of the first instar but larger in size. The cephalopharyngeal skeleton is thickened and less slender in form than that of the first instar. It resembles the cephalopharyngeal skeleton of the mature larva except that the posterior sinuses of the lateral pharyngeal

sclerites are much deeper, thus making the dorsal and ventral posterior processes more slender than in the mature larva. The second larval instar may undergo ecdysis in twenty-four hours at a temperature of 25°—35°C., but under cooler conditions or with a deficiency of moisture the period is prolonged and may take several days.

The third larval stage or third instar, which is the last larval stage, grows rapidly. The anatomy of this the mature larva will be fully described. Larvæ incubated at a temperature of 35°C. complete this larval stage and pupate in three to four days, on the other hand, under less favourable developmental conditions, it sometimes extended over a period of eight or nine days. Incubated larvæ cease feeding at the end of the second day of this stage and gradually assume a creamy colour, which colour is due to the large development of the fat body and to the histolytic changes which are taking place internally; larvæ dissected at this stage contain a very large amount of adipose tissue cells. Between the third and fourth day the larva contracts to form the pupa.

4. The Pupa.—The process of pupation may be completed in so short a time as six hours. The larva contracts, the anterior end especially being drawn in, with the result that a cylindrical pupal case is formed (fig. 15), the posterior region being very slightly larger in diameter than the anterior; the anterior and posterior extremities are evenly rounded. The average length of the pupa is 6·3 mm. Owing to the withdrawal of the anterior segments the anterior spiracular processes (*a.sp.*) are now situated at the anterior end, and the posterior spiracles (*p.sp.*) form two flat button-like prominences on the posterior end. The pupa changes from the creamy-yellow colour of the larva to a rich dark brown in a few hours. As the last larval skin has formed the pupal case, it being a coarctate pupa, in addition to the persistance of the spiracular processes the other larval features such as spiny locomotory pads can be seen.

During the first twelve hours or so of pupation the larva loses its tracheal system, which appears to be withdrawn

anteriorly and posteriorly, the latter moiety being the larger; the discarded larval tracheal system lies compressed against the interior of the pupal case (*l.tr.*). Communication with the external air is formed for the nymphal[1] developing tracheal system by means of a pair of temporary pupal spiracles, which appear as minute spine-like lateral projections between the fifth and sixth segments of the pupal case (*n.sp.*). Each of these communicates with a knob-like spiracular process (fig. 10, *n.sp.*) attached to the future prothoracic spiracle of the fly. The proctodæal and stomodæal portions of the alimentary tract are also withdrawn, and with the latter the cephalo-pharyngeal skeleton, which lies on its side on the ventral side of the anterior end of the pupal case.

The histogenesis of the nymph is extremely rapid, so that at the end of about thirty hours, in the rapidly developing specimens, it has reached the stage of development shown in fig. 10, in which most of the parts of the future fly can be distinguished although they are ensheathed in a protecting nymphal membrane. The head, which with the thorax has been formed by the eversion of the cephalic and thoracic imaginal discs from their sacs, is relatively large: two small tubercles (*an.*) mark the bases of the antennæ. The proboscis is enclosed in a large flat sheath which at this period appears to be distinctly divided into labral (*lbr.*) and labial (*lb.*) portions. In a short time the parts of the proboscis are seen to develop in these sheaths (fig. 11). The femoral and tibial segments of the legs are closely adpressed and lie within a single sheath. The wings (*w*) appear as sac-like appendages, and, as the nymphal sheath of the wing does not grow beyond a certain size, the wing develops in a slightly convoluted fashion by means of a fold which appears in the costal margin a short distance from the apex of the wing.

With a constant temperature of about 35° C., or even less, the exclusion of the imago may take place between the third

[1] The word "nymph" is used here to designate that stage in the development which begins with the appearance of the form of the future fly, and ends when the exclusion of the imago takes place.

and fourth day after pupation, but it is more usually four or five days as the larvæ, when about to pupate, leave the hotter central portion of the mass in which they have been feeding and pupate in the outer cooler portions : this outward migration may be a provision for the more easy emergence of the excluded fly from the larval nidus. In some cases the pupal stage lasts several weeks, but I have never succeeded in keeping pupæ through the winter.

When about to emerge, the fly pushes off the anterior end of the pupal case in dorsal and ventral portions by means of the inflated frontal sac, which may be seen extruded in front of the head above the bases of the antennæ. The splitting of the anterior end of the pupal case is quite regular, a circular split is formed in the sixth segment and two lateral splits are formed in a line below the remains of the anterior spiracular processes of the larva. The fly levers itself up out of the barrel-like pupa and leaves the nymphal sheath. With the help of the frontal sac which it alternately inflates and deflates it makes its way to the exterior of the heap and crawls about while its wings unfold and attain their ultimate texture, the chitinous exoskeleton hardening at the same time; when these processes are complete the perfect insect sets out on its career.

V. The Larva of Musca domestica.

1. External Features.—The external appearance of the typical acephalous muscid larva or "maggot" (fig. 5) is well known. It is conically cylindrical. The body tapers off gradually to the anterior end from the middle region. The posterior moiety is cylindrical, and except for the terminal posterior segment the segments are almost equal in diameter. The posterior end is obliquely truncate. The cuticular integument is divided by a number of rings; this ringed condition is brought about by the insertion of the segmentally-arranged somatic muscles the serial repetition of which can

be clearly understood by reference to fig. 16. The average length of the full-grown larva of M. domestica is 12 mm.

The question as to the number of segments which constitute the body of the muscid larva is a debated subject. I have, however, taken as my criterion the arrangement of the somatic musculature. Newport (1839) considered that the body of the larva of Musca vomitoria consisted of fourteen segments, but if the anterior portion of the third segment, that is, my first post-oral segment, is included, there were fifteen, to which view he appeared to be inclined. Counting the anterior segment or "head" as the first, Weismann (1863 and 1864) considers that the body is composed of twelve segments. Brauer (1883) is of the opinion that there are twelve segments, but that the last segment is made up of two; Lowne follows this view in his description of the blow-fly larva and considers that there are fifteen post-oral segments. I am unable to accept Lowne's view. Counting the problematical cephalic segment, for which I shall use Henneguy's (1904) term "pseudo-cephalon," as the first segment, I believe that it is succeeded by twelve post-oral segments, making thirteen body segments in all, which is the usual number for dipterous larvæ as Schiner (1862) has also pointed out. My study of the somatic musculature, as I shall show, indicates the duplicate nature of the apparent first post-oral segment, so that the apparent second post-oral segment (iv), that is, the segment posterior to the anterior spiracular processes, is really the third post-oral segment or fourth body-segment.

The cephalic segment cannot be considered as homologous with the remaining twelve segments, which are true segments of the body as shown by their musculature and innervation. This segment (fig. 9, i), for which Henneguy's term "pseudo-cephalon" is very suitable, probably represents a much reduced and degenerate cephalic segment, its present form being best suited to the animal's mode of life. We may consider the greater part of the cephalic segment of the larva as having been permanently retracted within the head; this is shown by

the position of the pharyngeal skeleton, to the whole of which the name "cephalo-pharyngeal skeleton" has been given. All that now is left of the cephalic segment consists of a pair of oral lobes, whose homology with the maxillæ is very problematical, and at present is not safely tenable. On the dorsal side the oral lobes are united posteriorly. Each bears two conical sensory tubercles (*o. t.*), which are situated, the one dorsally, and the other anterior to this and almost at the apex of the oral lobe. The ventral and ventro-lateral surfaces of the oral lobes are traversed by a number of channels, which will be described later.

The post-cephalic segment, which is composed of the first and second post-oral segments and represents the second and third segments of the body, is conical in shape. The first post-oral segment (ii), to which Lowne gave the name "Newport's segment," is limited posteriorly by a definite constriction and is covered with minute spines. The second post-oral segment bears laterally at its posterior border the anterior spiracular processes (*a. sp.*) The remaining segments of the body—four to twelve—are on the whole similar in shape. At the anterior edge of the ventral side of each of the sixth to twelfth body-segments there is a crescentic pad (fig. 5, *sp.*) bearing minute and recurved spines; these are locomotory pads by means of which the larva moves forwards and backwards. It is important to note that these pads are situated on the anterior border of the ventral side of each segment as they do not appear to have been carefully placed in the previous figures of this species. In addition to these spiniferous pads there are two additional pads of a similar nature, one on the posterior border of the ventral side of the twelfth body-segment, and the other posterior to the anus.

The terminal or thirteenth body-segment is obliquely truncate, but the truncate surface, which occupies more than half the posterior end of the larva, is not very concave as in the blow-fly larva. It bears in the centre the two posterior spiracles (fig. 3, *p. sp.*), which are described in detail with the tracheal system. On the ventral side of the terminal segment

are two prominent anal lobes (fig. 5, *an. l.*), which are important agents in locomotion.

The cuticular integument is thin and rather transparent, so that in the younger larvæ many of the internal organs can be seen through it. In older larvæ the fat-body assumes large proportions and gives the larva a creamy appearance, obscuring the internal organs. The cuticle (fig. 14) is composed of an outer rather thin layer of chitin (*ct.*), which is continuous with the chitinous intima of the tracheæ, and also with the chitinous lining of the stomodæal and proctodæal regions of the alimentary tract. Below this layer there is a thicker layer of chitin (*ct.'*), which does not stain so deeply. In places this lower layer is penetrated by the insertions of the muscles. The cuticle lies on a layer of stellate hypodermal cells (*hy.*), which are well innervated, and attain a large size in the posterior segments of the body.

2. Muscular System.

The muscular system of the larva (Pl. 31, fig. 16) consists of a segmental series of regularly repeated cutaneous muscles, forming an almost continuous sheath beneath the skin, together with a set of muscles in the anterior segments of the body which control the cephalo-pharyngeal sclerites and pharynx. In addition to this there are a set of cardiac muscles and the muscles of the alimentary tract.

I have been unable to find a detailed description of the muscular system of the muscid larva, and I do not think that Lowne's excuse for dismissing the cutaneous muscles of the blow-fly larva with a very brief statement, because "the details possess little or no interest," was justified, considering how little is known about the muscular systems of insect larvæ, and constant reference to the classic work of Lyonet (1762) on the caterpillar is not sufficient to satisfy the inquiring student of to-day. The muscular system of the larva, therefore, will be described in some detail.

Muscles of the body-wall.—The cutaneous muscles

are repeated fairly regularly from segments (by segments I mean body-segments) four to twelve and a detailed description of the muscles of one of these segments will serve for the rest. The muscles, though continuous in most cases from segment to segment, are attached to the body-wall at the junction of the segments. The most prominent muscles are the dorso-lateral oblique recti muscles. In segments six to twelve there are four pairs each of external (*ex. d. l.*), and internal dorso-lateral oblique recti (*in. d. l.*) muscles; in segments four and five there are five pairs of external and six pairs of internal dorso-lateral oblique muscles. Ventral to these muscles are four pairs of longitudinal ventro-lateral muscles (*l. v. l.*); the muscle bands of the two more ventral pairs are double the width of those of the two more lateral pairs. In the fifth segment there is only one of the more lateral pairs of the longitudinal ventro-lateral muscles present, and in the fourth segment only the two more ventral pairs remain. In addition to these muscles there are two other pairs of oblique recti muscles; these are, a pair of ventro-lateral oblique muscles (*v. l. o.*) and a pair of internal lateral oblique muscles (*i. l. o.*); both of these are absent in the segments anterior to the sixth. The foregoing muscles, namely the dorso-lateral oblique, the internal lateral oblique, the ventro-lateral oblique and the longitudinal ventro-lateral, by their contraction, bring together the intersegmental rings and so contract the body of the larva.

Attached externally to the anterior ends of the longitudinal ventro-lateral muscles are a number of pairs of ventral oblique muscles (*v. o.*); they vary in number from two to eight pairs in each segment. The number increases posteriorly from two pairs in segment four to four pairs in segment five, five pairs in segment seven, seven pairs in segment ten, eight pairs in segment eleven; the number of pairs then decreases to six or seven pairs in segment twelve. The more ventral pairs of these muscles are not attached at their posterior ends to the intersegmental ring but to the ventral wall of the segment and no doubt assist in bringing forward the ventral spiniferous pads. In segments four to twelve there are three pairs of

lateral muscles (*l. m.*) situated next to the hypodermis and attached in a dorso-ventral position; these will assist in drawing the dorsal and ventral regions of the segments together and so increase the length of the larva. Between segments four and five and the remaining segments to twelve there is, on the intersegmental ring, a pair of lateral intersegmental muscles (*l. i. m.*); these by their contraction bring about a decrease in the size of the intersegmental ring and so assist the lateral muscles in increasing the length of the larva.

The muscles of the last segment (xiii) are not regularly arranged as in the preceding segments; they consist of three main groups: (1) the recti muscles, which assist in contracting the segments; (2) the anal muscles (*an. m.*), which are attached ventrally to the anal lobes (*an. l.*); and (3) the dorso-ventral muscles (*d. v.*), which by their contraction assist in lengthening the segment. In addition to these there are certain small muscles in relation with the posterior spiracles.

In the second and third segments the recti muscles are reduced to four pairs and the attachment of the two lateral and external pairs of muscles has led me to regard the apparently single first post-oral segment as consisting of two segments; it is not a single post-cephalic or pro-thoracic segment as it has been called. There is quite a distinct internal division and the external constriction has been already noticed. This view does not necessarily alter the homology of the third segment, which may still be regarded as pro-thoracic if this is desirable. The segment which I regard as the second body-segment may be a rudiment of the cephalic region which has been almost lost, and this loss, or, as I prefer to regard it, this withdrawal of the head, only serves to make any discussion as to the homologies of these anterior segments with those of the adult extremely difficult, and, I believe, at present valueless. Further, comparative studies of the larvæ of the calyptrate muscidæ are necessary before we can arrive at any definite conclusions concerning the composition of the bodies of these larval forms.

The cephalo-pharyngeal muscles (fig. 19).—These muscles consist of four sets: (1) The cephalic retractor muscles, which by their contraction draw the anterior end of the larva and the pharyngeal mass inwards; (2) the protractor and depressor muscles of the pharyngeal mass; (3) the muscles controlling the mandibular, dentate, and hypostomal sclerites; and (4) the internal pharyngeal muscles.

There are four chief pairs of cephalic retractor muscles, of which the two ventral pairs are by far the largest. The more ventral of these two pairs (*v'. c. r.*) arises on the ventral side from the posterior end of the sixth segment, internal to the ventro-lateral longitudinal muscles; the other pair (*v. c. r.*), which is double, arises more laterally from the posterior end of the fifth segment. The remaining pairs of cephalic retractors arise from the posterior end of the third segment. All the cephalic retractor muscles are inserted anteriorly into a ring, the cephalic ring (*c. r.*), on the anterior border of the second segment, the first post-oral segment.

There are two pairs of cephalo-pharyngeal protractor muscles, a dorsal (*d. c. p.*) and a ventral pair (*v. c. p.*). Both are rather broad fan-shaped muscles inserted by their broad ends in the middle of the third segment, slightly to the sides of the dorsal and ventral median lines respectively. The dorsal and ventral muscles of each side are inserted together on the dorso-lateral region of the posterior end of the pharyngeal mass. The pair of depressor muscles (*d. m.*) which are situated dorsally, are attached by their broader ends to the intersegmental ring between segments three and four. They are inserted on to the posterior end of the dorsal side of the pharyngeal mass; by their contraction the posterior end of the pharyngeal mass is raised, the result being that the sclerites articulated to its anterior end are depressed.

There remain six pairs of muscles controlling the mandibular, dentate and hypostomal sclerites, one pair controlling the two foremost sclerites and four pairs controlling the hypostomal sclerite. The mandibular extensor muscles (*m. e.*) are attached to the body-wall in the third segment on each side

of the median line and between the dorsal cephalo-pharyngeal protractors. They are inserted on to the dorsal side of the mandibular sclerite (*m. s.*); by their contraction they elevate the sclerite. This sclerite is depressed by the contraction of a pair of muscles which control the dentate sclerites (*d. s.*), the latter fitting into a notch on the ventral side of the mandibular sclerite. The mandibular depressor muscle (*m. d.*) is attached to the posterior ventral process of the lateral pharyngeal sclerite by the three bands into which the posterior portion of the muscle is divided; the anterior and single end of the muscle is inserted on the ventral process of the dentate sclerite. Four pairs of muscles (*s. d.*) are inserted on the hypostomal sclerite (*h. s.*). Two more dorsal pairs are attached to the intersegmental ring between segments three and four as shown in fig. 16. The two more ventral pairs are attached to the lateral pharyngeal sclerites, one being attached to the ventral side of the posterior dorsal process and the other to the ventral process beneath the mandibular depressor. These muscles, which I call the stomal dilators, are inserted on the sides of the hypostomal sclerite. Their function is, I believe, to open and close the anterior pharyngeal aperture and so control the flow of fluid food into the pharynx and of the salivary secretion; the lowest pair of muscles may be more directly concerned with the latter.

The pharyngeal apparatus is controlled, as in the adult fly, by a series of muscles. In the larval stadium, however, where so large an amount of food is required for the growth and building up of the future insect, there is a greater development and elaboration of the pharyngeal apparatus, including the muscles. In the greater anterior region of the pharynx, that is, the part lying within the pharyngeal sclerites (fig. 18), the muscular system consists of two bands of oblique muscles (*o. ph.*) arranged in pairs. The muscles are attached dorsally to the inside dorsal edges of the lateral plates (*l. p.*) and ventrally to the roof of the pharynx (*r. ph.*), the ventral attachment being more posterior than the dorsal. The posterior region of the pharynx, which is between the lateral plates and

the œsophagus (fig. 17), is controlled by two sets of muscles. Two pairs of elongate oblique muscles (*e. o. m.*) are attached dorsally to the dorsal edges of the lateral plates (*l. p.*) and inserted ventrally on to the roof of the pharynx ; these muscles assist the previously described oblique pharyngeal muscles in raising and depressing the roof of the pharynx. They are assisted in enlarging and contracting the lumen of the posterior part of the pharynx by a number of semi-circular dorsal muscles (*s. d. m.*), which by their contraction make the floor of the pharynx more concave, and it is these muscles, I believe, that are chiefly concerned in the maintenance of the peristaltic contractions of the pharynx, by means of which the fluid food, which has been sucked into the mouth by the pumping action of the pharynx, is carried on to the œsophagus.

The similarity between the pharyngeal apparatus of the fly, that is, of the fulcrum and that of the larva, is very striking, both with regard to the form of the skeletal structures and the musculature. If the pharynx of the larva were regarded as being homologous to that of the fly it would further support the view that the head of the larva had been permanently withdrawn into the succeeding anterior body-segments. These structures, however, may be merely analogous ; the similarity of structure may have been brought about by similarity of function. Both larva and adult subsist on fluids which are sucked into the mouth and pumped into the œsophagus.

The series of muscular actions which takes place during locomotion appears to be as follows. By the contraction of the pharyngeal protractors the anterior end of the larva is extended, the mandibular sclerite being extended at the same time by the contraction of the mandibular extensor muscles. The mandibular sclerite is now depressed by the contraction of the mandibular depressors, and anchors the anterior end of the larva to the substance through which it is moving. A series of segmental linear contractions now takes place, initiated by the large cephalic retractor muscles, and carried on posteriorly from segment to segment by the

dorso-lateral oblique, the internal lateral oblique, the longitudinal ventro-lateral, the ventro-lateral oblique and ventral oblique muscles. Each segment as it comes forward takes a firm grip ventrally by means of the spiniferous pad. By the time the last spiniferous pad has become stationary the mandibular sclerite has left its anchorage, and by the contraction of the lateral and intersegmental muscles, which takes place from before backwards, the lengths of the segments of the larva are increased serially and the anterior end begins to move forward again, when the whole process is repeated.

3. Nervous System.

The central nervous system of the larva (Pl. 32, fig. 23) has attained what would appear to be the limit of ganglionic concentration and fusion. The boat-shaped ganglionic mass, which lies partly in the fifth segment, but the greater portion in the sixth segment, is a compound ganglion and represents the fusion of eleven pairs of ganglia similar to that which Leuckart (1858) describes in the first larval stage of Melophagus ovinus, but which, however, has not undergone so great a degree of concentration as in M. domestica. This ganglionic mass, which for convenience and brevity I shall call the ganglion (Lowne's "neuroblast") does not exhibit externally any signs of segmentation, the interstices between the component ganglia being filled up with the cortical tissue, whose outer wall forms a plain surface. In horizontal and sagittal sections, however, the component ganglia can be recognised and their limits are more clearly defined. The ganglion is surrounded by a thick ganglionic capsular sheath which is richly supplied with tracheæ, and appears to be continuous with the outer sheath of the peripheral nerves. Two pairs of large tracheæ (fig. 24) are found entering the ganglionic sheath, an anterior pair (*tr.'*) which runs in between the cerebral lobes, and a lateral pair (*tr.''*) entering the ganglion beneath these lobes. In the young larva the cortical

layer of cells is proportionately much thicker. The cortical tissue is made up of cells of varying sizes, but which can be grouped in two classes—smaller cortical cells and larger ganglionic cells. Most of the ganglionic cells appear to be unipolar, but there are many of a bipolar and multipolar nature present; they stain readily and possess fairly large nuclei. These ganglionic cells are arranged segmentally, and occur near the origin of the nerves. In the posterior region of the ganglion, where the nerves arise in close proximity, the ganglion cells are very numerous, relatively few of the cortical cells being found. A further demarcation of the component ganglia is brought about by median and vertical strands of the ganglionic sheath-tissue, which perforate the compound ganglion and occur as vertical strands along its median line. Tracheæ also penetrate the ganglion with these strands of capsular tissue.

On the dorsal side of the anterior end of the ganglion is situated a pair of spherical structures (*c. l.*), which may be termed the "cerebral lobes." They are united in the median line dorsal to the foramen traversed by the œsophagus (*oe.*). These cerebral lobes are chiefly of an imaginal character, and contain the fundaments of the supra-œsophageal ganglia and also of the optic ganglia of the future fly (fig. 27). Each is surrounded by a thin membranous sheath (*sh.*) and is connected with the major cephalic imaginal discs by the optic stalk (*o. s.*).

The nerves arising from the ganglion may be divided into three groups, according to their origin. Eleven pairs of nerves (fig. 24, 1–11) corresponding to the eleven pairs of ganglia arise, two from the anterior end and nine from the sides of the ganglion. Three pairs of nerves (*a.*, *b.* and *c.*) arise laterally from the stalks of the pro-thoracic and meso-thoracic imaginal discs. In the median dorsal line of the posterior half of the ganglion a single pair (*d. a.'*) and two median unpaired (*d. a.''* *d. a.'''*) nerves have their origin; these are accessory nerves.

The first pair of the two anterior pairs of nerves runs

forward and innervates the posterior region of the pharyngeal mass; the anterior region of the latter is supplied by the second pair of nerves. These nerves also innervate the anterior segments of the body. The first (*a*) of the three pairs of nerves which arise from the stalks of the imaginal discs runs to the anterior end supplying the protractor and retractor muscles of the pharyngeal mass. The second (*b*) of these three pairs of nerves innervates the muscles of the body-wall of the third and fourth segments; the latter segment is also innervated by the third (*c*) of the three pairs of nerves. The succeeding nine pairs of lateral nerves are segmentally distributed, and innervate the muscles of the body-wall of segments five to thirteen. Each nerve bifurcates on reaching the muscles, and these branches further subdivide into very fine nerves.

The nerves, which arise dorsally, and which I have called the accessory nerves, are interesting. The first pair (*d. a.'*) which arises about mid-way along the dorsal side of the ganglion, accompanies the pair of nerves supplying the seventh segment. The second (*d. a.''*), which is an unpaired nerve, bifurcates in the seventh segment, and the resulting nerves proceed to the body-wall in association with the nerves supplying the eighth segment. The third and posterior dorsal accessory nerve (*d.a.'''*) bifurcates in the seventh segment. Each of the resulting nerves undergoes a second bifurcation; the dextral nerve, bifurcating in the eighth segment, accompanies the nerves supplying the ninth segment; the sinistral nerve bifurcates between segments eight and nine, and the resulting nerves proceed to the tenth segment. None of the remaining lateral nerves appear to be accompanied by an accessory nerve, of which there are four pairs only. The ganglionic sheath is penetrated by tracheæ, some of which arise from the ganglion in association with the nerves which they accompany to the body-wall. Two of these tracheæ are shown (fig. 24, *t.*). Similar fine tracheæ arise with the three posterior pairs of lateral nerves, and on account of their similarity to accessory nerves I at first

mistook them for such, even when dissecting with a magnification of sixty-five diameters, until my serial sections showed their real nature. Without sections it is impossible to distinguish these fine unbranching tracheæ from accessory nerves. I have mentioned this fact as showing the necessity of supplementing the one method by the other.

The visceral or stomatogastric nervous system (Pl. 31, fig. 20) consists of a small central ganglion (*c. g.*) lying on the dorsal side of the œsophagus, immediately behind the transverse commissure of the cerebral lobes from the bases of which two fine nerves are given off to join a fine nerve from the ganglion, which runs dorsally towards the anterior end of the dorsal vessel. A fine nerve from the ganglion runs forward on the dorsal side of the œsophagus towards the pharynx. A posterior nerve (fig. 24, *v.n.*) runs from the ganglion along the dorsal side of the œsophagus to the neck of the proventriculus, where it forms a small posterior ganglion (fig. 20, *pv. g.*), from which fine nerve-fibres arise and run over the anterior end of the proventriculus.

Sensory organs.—The only sensory organs which the larva possesses are the two pairs of conical tubercles (fig. 9, *o. t.*), which have been described already on the oral lobes. In section each consists of an external transparent sheath of the outer cuticular layer; beneath this and surrounded by a chitinous ring are the distal cuticularised extremities of a number of elongate fusiform cells grouped together to form a bulb. These are nerve-end cells and their proximal extremities are continuous with nerve-fibres by means of which they are connected to the ganglion. Both sensory organs of each oral lobe are supplied by the same nerve from the second of the two anterior nerves. Judging from their structure these organs appear to be of an optical nature, and this is the usual view which is held with regard to their function. They would appear merely to distinguish light and darkness, which, for such cryptophagous larva, is no doubt all that is necessary. The negative heliotropism of the larva of the blow-fly has been experimentally proved by Loeb (1890), and my own

observations confirm the same for the larvæ of M. domestica.

The hypodermal cells are well innervated and the body-wall appears to be highly sensitive.

4. The Alimentary System.

The alimentary tract increases in length at each of the larval ecdyses, and in the mature larva (Pl. 33, fig. 29), its length is several times greater than the length of the larva. The great length of the alimentary tract of the larva compared with that of the fly is probably accounted for by the fact that a large digestive area is necessary for the rapid building up of the tissues from fluid food which takes place during the larval life. It is divisible into the same regions as the alimentary tract of the mature insect, but it differs from the latter in several respects; these regions are parts of the original stomodæal, mesenteric and proctodæal regions of which the mesenteric is by far the longest in this larva. The regions of the alimentary tract which are derived from the stomodæum and proctodæum are lined with chitin of varying thickness which is attached during life to the epithelial cells, but is shed when the larva undergoes ecdysis. The mesenteron does not appear to be lined with chitin as it is in some insects, in which cases the chitinous intima usually lies loose in the lumen; it is, however, in the larva of M. domestica, usually lined with a lining of a mucous character. The whole alimentary tract is covered by a muscular sheath of varying thickness.

The mouth (fig. 6, *m.*) opens on the ventral side between the oral lobes. The ventral and ventro-lateral sides of the oral lobes are traversed by a series of small channels (fig. 14, *ch.*), which are made more effective by the fact that one side of the channel is raised and overhangs the other so as to partially convert the channels into tubes rather comparable to the pseudo-tracheæ of the oral lobes of the fly, to which they have a similar function: the liquid food runs along these

channels to the mouth. Distally many of the channels unite ; the resulting channels all converge and run into the mouth. The anterior border of the oral aperture is occupied by the mandibular sclerite (*m. s.*), and the posterior border is bounded by a lingual-like process (*l.*) that is bilobed at its anterior extremity.

Cephalo-pharyngeal sclerites (Pl. 30, fig. 4).—The sclerites associated with the cephalo-pharyngeal region are rather similar to those of the second larval instar; they are, however, of a more solid and of a thicker character. Between the oral lobes is seen the median uncinate mandibular sclerite (*m. s.*). The homology of this sclerite is obscure. Lowne regarded it as being the labrum ; some authors consider that it represents the fused mandibles. As we know at present so little of the comparative embryology of these larvæ it will be best to retain the name by which it is generally known. The basal extremity of the mandibular sclerite is broad, and at each side a dentate sclerite (*d. s.*) is articulated by means of a notch in the side of the mandibular sclerite, the function of which has been shown already in describing the muscles. The mandibular sclerite articulates posteriorly with the hypostomal sclerite (*h. s.*). This consists of two irregularly-shaped lateral portions united by a ventral bar of chitin ; it is anterior to this bar of chitin that the salivary duct opens into the front of the pharynx. The sides of the hypostomal sclerite articulate with two processes on the anterior edge of the lateral pharyngeal sclerites (*l. p.*). The lateral pharyngeal sclerites or plates recall the shape of the fulcrum of the adult fly. Each is wider posteriorly than anteriorly, and the posterior end is deeply incised ; at the base of this incision the nerves and tracheæ which supply the interior of the pharynx enter. The lateral sclerites vary in thickness, as will be seen in the figures of the sections of the pharynx. They are united dorsally at the anterior end by a dorsal sclerite (*d. p. s.*), and ventrally they are continuous with the floor of the pharynx.

The pharynx (Pl. 31, figs. 17 and 18) in certain respects is

similar to that portion of the pharynx of the fly which lies in the fulcrum. The whole length of the floor of the pharynx is traversed by a series of eight grooves separated by bifurcating ribs which are T-shaped in section (fig. 18, *t. r.*), and are called the "T ribs" by Holmgren (1904); they form a series of eight tubular grooves. Holmgren believes that they may have been derived from a condition similar to that found in the pharynx of the larva of Phalacrocera, where the floor of the pharynx is traversed by a number of deep but closed longitudinal fissures. These pharyngeal grooves probably have a straining function, but they may also be of use in allowing a certain amount of the salivary secretion to flow backwards towards the œsophagus. The musculature and action of the pharynx has been described. On the dorsal side of the pharyngeal mass and attached laterally to the layer of cells covering the lateral sclerites there is a loose membrane (*m.*), whose function, I believe, is to accommodate the blood contained in the pharyngeal sinus (*p.s.*) when the roof of the pharynx is raised. Posteriorly the floor of the pharynx curves dorsally and opens into the œsophagus.

The œsophagus (fig. 29, *œ.*) is a muscular tube beginning at the posterior end of the pharyngeal mass. It describes a dorsal curve when the larva is contracted, and then runs in a straight line through the œsophageal foramen between the cerebral lobes of the ganglionic mass and dorsal to the ganglion to the posterior region of the sixth larval segment, where it terminates and opens into the proventriculus. It is of a uniform width throughout and is lined by a layer of flat epithelial cells (fig. 25, *œ. ep.*) whose internal faces are lined by a chitinous sheath (*ch. i.*), which is thrown into a number of folds. There is nothing of the nature of a ventral diverticulum forming a crop such as Lowne describes in the larva of the blow-fly.

The proventriculus (fig. 29, *pv.*) varies slightly in shape according to the state of contraction of the alimentary tract; in the normal condition it is cylindrically ovoid and its axis is parallel with that of the body. As will be seen from the

figure (fig. 25), it is rather similar to the proventriculus of the imago in general structure. The œsophageal epithelium penetrates a central core which is composed of large clear cells (*c. c.*); its lumen, being œsophageal, is lined with chitin. This core is surrounded by an outer sheath, the cells (*e. v.*) of which are continuous with those of the ventriculus. At the junction of the central core with the outer sheath of cells there is a ring of small more deeply-staining cells (*i. c.*). This ring was regarded by Kowalevski (1887) as the rudiment of the stomodæum of the nymph, but Lowne is of the opinion that it develops in the nymph into the proventriculus of the imago. I believe that it forms a portion, at least, of the proventriculus of the imago, as it exhibits a very close resemblance to the ring of cells in this region figured in the section of the proventriculus of the imago (fig. 20 of Part I).

The mesenteron of the mature larva is of very great length, and is not divisible into the two regions of ventriculus and small proximal intestine as in the imago, but appears to have the same character throughout; hence Lowne calls it the "chyle-stomach," which term, or ventriculus (fig. 29, *v.*), may be used to designate the whole region from the proventriculus to the point at which the malpighian tubes arise. It is very much convoluted and twisted upon itself. The course of the ventriculus is almost constant, and can be better understood from the figure than from any detailed description. At the anterior end four tubular cæca (*c. v.*) arise. Their walls consist of large cells whose inner faces project into the lumen of the glands; these glands were not present in the imago. The epithelium of the ventriculus (fig. 30) is composed of large cells (*e. v.*), which project into the lumen of the alimentary tract; they possess large nuclei and the sides of the cells facing the lumen have a distinct striated appearance, which is absent in those epithelial cells covered with a chitinous intima. This striated appearance may be related in some way to the production of the mucous intima which is generally present in the ventriculus, and which appears to take the place of the loose chitinous intima or peritrophic

membrane which occurs in this region in numerous insects, and which has been studied in detail by Vignon (1901) and others. Below the epithelial cells a number of small cells (*g. c.*) are found, which may be either gland cells or young epithelial cells. In addition to these cells small groups of deeply-staining fusiform cells (*i. c.*) are found below the epithelium. These, I believe, are embryonic cells from which the mesenteron of the imago arises. The Malpighian tubes arise in the tenth segment at the junction of the ventriculus and the intestine.

The intestine (fig. 29, *int.*) is narrower than the ventriculus and runs forwards as far as the eighth segment, where it bends below the visceral mass and runs posteriorly, to become dorsal again behind the tenth segment, from whence it runs backwards, turning ventrally behind the visceral mass to become the rectum. The epithelium is thrown into a number of folds and is covered with a chitinous intima.

The rectum (*r.*) is very short and muscular, and the chitinous intima is fairly thick and continuous with the outer cuticular layer of the chitinous integument. It is almost vertical and opens by the anus on the ventral side of the terminal larval segment between the two swollen anal lobes.

Salivary glands.—There is a pair of large tubular salivary glands (*s. gl.*) lying laterally in segments five and six. Anteriorly each is continued as a tubular duct; the two ducts approach each other and join beneath the pharyngeal mass to form a single median duct (fig. 19, *sal. d.*) which runs forward and opens into the pharynx on the ventral side as already described. The glands are composed of large cells (fig. 21), which project into the lumen of the gland; they stain deeply and have large active nuclei. The salivary secretion, apart from the digestive properties which it has, is no doubt of great importance in making the food more liquid, as is also the case in the imago, and so rendering it more easy for absorption.

The Malpighian tubes (fig. 29, *m. t.*) arise at the junction of the ventriculus and intestine in the tenth segment. A short

distance from their origin they bifurcate and the resulting four tubules have a convoluted course, being mingled to a great extent with the adipose tissue. They are similar in appearance and histologically to those of the imago, consisting of large cells, of which only two can be seen usually in section; they consequently give the tubules a moniliform appearance. In the mature larva these cells appear to break down to form small deeply-staining spherical bodies. This histological degeneration begins at the distal ends of the tubules, which in the mature larva usually have the appearance shown in fig. 28 (*m. t.*); all the stages of degeneration can be traced out. This process may be a means of getting rid of the remaining larval excretory products.

The four cæca at the anterior end of the ventriculus have already been described.

5. The Respiratory System.

The tracheal system (fig. 26) of the adult larva consists of two longitudinal tracheal trunks united by anterior and posterior commissures, and communicating with the exterior by anterior and posterior spiracles, the latter are situated in the middle of the oblique caudal end, and the anterior spiracles, which are not present in the first larval instar, are situated laterally at the posterior border of the third body-segment.

I believe that the anterior spiracles (*a. sp.*) are true functional spiracles, though for some time I shared Lowne's opinion that they were not functional. This latter view was due to the fact that it was difficult to understand how these spiracles could obtain air when they are immersed, as they usually are, in the moist fermenting materials on which the animal feeds. A careful examination of their structure, however, strengthens my belief that they are able, if necessary, to take in air; the occasions when this is possible are probably not infrequent. Each of the anterior spiracular processes consists of a fan-shaped body (fig. 9, *a. sp.*) bearing six to eight small papilliform processes. The papillæ (fig. 7)

open to the exterior by a small pore which leads into a cavity having a clear lumen surrounded by branched cuticular processes, whose function is probably to prevent solid particles from penetrating the spiracular channel. The body of the fan-shaped spiracular process is filled with a fine reticulum of the chitinous intima, which Meijere (1902) calls the "felted-chamber" (Filzkammer); through this meshwork the air can pass to the longitudinal tracheal trunk.

The posterior spiracles (fig. 3, *p. sp.*) are D-shaped with the corners rounded off and their flat faces are opposed. Each consists of a chitinous ring having internal to the flat side a small pierced knob. Each chitinous ring encloses three sinuous slits, guarded by inwardly-directed fine dendritic processes; through these slits the air enters the small spiracular atrium, one of which is situated internal to each of the spiracles. The spiracular atria communicate directly with the longitudinal tracheal trunks.

The course and origin of the branches of each of the longitudinal tracheal trunks (fig. 26 *l. tr.*) is the same, so that of the left side will be described only. Immediately behind the spiracular atria the short posterior tracheal commissure (*p. com.*) connects the two trunks. In the younger larvæ this commissure is situated more anteriorly, but in the adult it is situated so far back and so close to the spiracles that its presence might easily be overlooked. On the outer side of the tracheal trunk a large branch arises; this, the visceral branch (*v. tr.*), bends ventrally to the lateral trunk, and thus becoming internal to it enters the convoluted visceral mass with its fellow of the other side. The visceral branches extend anteriorly as far as the seventh segment. In the twelfth and thirteenth segments the lateral tracheal trunk has a double appearance. A dorsal and a ventral branch arise in most of the segments, the dorsal branch chiefly supplies the fat body, and the ventral branch supplies the viscera; both give off branches to the muscular body wall. The anterior commissure (*a. com.*) is situated in the fourth segment. It crosses the œsophagus immediately behind the pharyngeal mass. On

the internal side of the portion of the lateral tracheal trunk that is anterior to the commissure a branch arises, and running ventral to the pharyngeal mass it supplies the anterior end of the larva and the oral lobes. A branch that supplies the muscles of this region is given off external to the origin of the anterior commissure. Internal to the origin of the commissure two tracheæ arise; the anterior branch enters and supplies the pharyngeal mass, and the posterior branch (*tr.'*) enters the ganglion ventral to the cerebral lobes. In the fifth segment another internal tracheal branch enters the ganglion (*tr.''*). These tracheæ which supply the ganglion appear to run chiefly in the peripheral regions, where they divide into a number of branches, the fate of some of these being interesting. These branches are extremely fine, and they arise, as I have previously mentioned, in association with a number of the segmental nerves with which they run to the body wall.

6. The Vascular System and Body Cavity.

The relations and structure of the vascular system of the larva are on the whole similar to those of the fly; there are, however, a number of modifications.

The dorsal vessel, which includes the so-called "heart," is a simple muscular tube lying on the dorsal side immediately beneath the skin, and extending from the posterior tracheal commissure to the level of the cerebral lobes of the compound ganglion in the fifth segment. Its wall is composed of fine striated muscle-fibres arranged transversly and longitudinally, but chiefly in the latter direction. The swollen posterior region (Pl. 33, fig. 31), which is called the heart, lies in the last three or four segments, its anterior limit being hard to define. It consists of three distinguishable chambers, which, however, are not divided by septa. Three pairs of ostia (*os.*), each provided with a pair of internal valves (*v.*), are situated laterally, and place the cardiac cavity in communication with the pericardium, in which this portion of the dorsal vessel lies. There are three pairs of alar muscles controlling the

action of this posterior cardiac region of the dorsal vessel. Lowne describes other openings in the wall of the "heart" of the blow-fly larva, but I have been unable to find others than those already described in this larva; it has three pairs only.

The dorsal aorta is the anterior continuation of the dorsal vessel, which gradually diminishes in diameter. When it reaches the fifth segment and lies above the ganglion, it terminates in a peculiar cellular structure (fig. 24, *c. r.*), which in the blow-fly has a circular shape and was called by Weismann the "ring." In the larva of M. domestica it has not so pronounced a ring-like appearance, but is more elliptically compressed and rather Λ-shaped. The cells of which it is composed have a very characteristic appearance, and are rather similar to a small group of cells lying on the neck of the proventriculus and at the anterior end of the dorsal vessel of the fly. From the lower sides of this cellular structure (fig. 28, *c. r.*) the outer sheaths of the major cephalic imaginal discs depend, and extend anteriorly to the pharyngeal mass, enclosing between them the anterior portion of the great ventral blood sinus.

The pericardium lies in the four posterior segments of the body, and is delimited ventrally from the general body-cavity by a double row of large characteristic pericardial cells. These cells have a fine homogeneous structure and are readily distinguished from the adjacent adipose tissue cells, whose size they do not attain. The pericardial cavity contains a profuse supply of fine tracheal vessels which indicates a respiratory function. A similar condition occurs in the blow-fly larva, and Imms (1907) has described a rich pericardial tracheal supply in the larva Anopheles maculipennis, as also Vaney (1902) and Dell (1905) in the larva of Psychoda punctata. The adipose tissue cells (fig. 28, *f. c.*) form the very prominent "fat-body." They are arranged in folded cellular laminæ that lie chiefly in the dorso-lateral regions of the body, and in section have the appearance shown in the figure. The cells have a similar structure to those of the adult fly; they are

very large, with reticular protoplasm containing fat globules, and there may be more than one nucleus in a single cell. As in the fly, the fat-body is closely connected with the tracheal system by means of a very rich supply of tracheæ.

Two chief blood-sinuses can be distinguished—the pericardial sinus, which has already been described, lying in the dorsal region in the four posterior segments, and the great ventral sinus. The latter lies between the outer sheaths of the major cephalic imaginal discs and extends anteriorly into and about the pharynx; posteriorly it encloses the ganglion and the convoluted visceral mass, above which it opens into the pericardial sinus between the pericardial cells.

The blood which fills the heart and sinuses and so bathes the organs is an almost colourless, quickly coagulable fluid, containing colourless, nucleated, amœboid corpuscles and small globules of a fatty character.

7. The Imaginal Discs.

As in other cyclorrhaphic Diptera, the imaginal discs of some of which have been described by Weismann (1864), Kunckel d'Herculais (1875–78) and Lowne, the imago is developed from the larva by means of these imaginal rudiments, which are gradually formed during the later portion of the larval life. They do not all appear at the same time, for whereas some may be in a well-developed state early in the third larval instar, others do not appear until the larva reaches its resting period or even later. The imaginal discs appear to be hypodermal imaginations though their origin is difficult to trace in all cases; in many instances they are connected with the hypodermis by means of a stalk of varying thickness. The imaginal disc or rudiment may consist of a simple or of a folded lamina of deeply-staining columnar embryonic cells, as in the wing discs, or of a number of concentric rings of these cells, as in the antennal and crural discs. They are usually closely connected with the tracheæ and in some cases are innervated by fine nerves. Although the imaginal discs

of M. domestica are similar in some respects to those of the blow-fly, as described by Lowne, there are several important differences, chief of which is the position of the imaginal discs of the meta-thoracic legs.

During the resting period of the larva the cephalic and thoracic discs can be distinguished, but the abdominal discs are small and not so obvious except in sections.

The cephalic discs.—The chief cephalic discs are contained in what at first appears to be a pair of cone-shaped structures in front of each of the cerebral lobes of the ganglion (fig. 24, *m. c. d.*) ; the cone, however, is not complete. The outer sheath of each of these major cephalic imaginal rudiments is continued dorsally, and joins the cellular structure mentioned previously (see fig. 28), thus enclosing a triangular space which is a portion of the ventral sinus. These sheaths are continued anteriorly and are connected to the pharyngeal mass, and it is through this connecting strand of tissue that the discs are everted to form the greater part of the head of the nymph. Immediately in front of the cerebral lobe is the so-called optic disc (fig. 27, *o. d.*), which in its earlier stages is cup-shaped, but later it assumes a conical form, having a cup-shaped base adjacent to the cerebral lobe. The optic disc is connected to the cerebral lobe laterally by a stalk of tissue, the optic stalk (*o. s.*), which becomes hollow later, and it is through this stalk that the optic ganglion and associated structures contained in the cerebral lobe appear to evaginate when the final metamorphosis and eversion of the imaginal rudiments takes place. The optic discs form the whole of the lateral regions of the head of the fly. The remaining portion of the head-capsule of the fly is formed from two other pairs of imaginal rudiments, the antennal and facial discs. The antennal disc (*an. d.*) lies in front of, and internal to, the optic discs. Each consists of an elongate conical structure, in which at a later stage the individual antennal joints can be distinguished. The facial discs (*f.d.*) are anterior to the antennal discs and extend to the anterior end of the conical structure containing these

three pairs of major cephalic discs, which will form the cephalic capsule.

In addition to these two other pairs of cephalic discs are found. A pair of small flask-shaped maxillary rudiments are situated one at the base of each of the oral lobes; a second pair of imaginal discs, similar in shape to the maxillary discs, is found adjacent to the hypostomal sclerite; the latter, I believe, are the labial rudiments, and will form almost the whole of the proboscis of the fly.

The thoracic discs.—In M. domestica there are five pairs of thoracic discs. The pro-thoracic imaginal discs (figs. 24 and 28, *pr. d.*) are attached to the anterior end of the ganglion and slope obliquely forwards; the distal end of each is attached to the body-wall on the ventral side between segments three and four. These discs develop into the pro-thoracic legs, and probably also into the much reduced pro-thoracic segment, as I was unable to discover any other rudiments corresponding to the dorsal imaginal discs of the meso-thoracic and meta-thoracic segments. Arising from the sides of the ganglion immediately behind the attachment of the pro-thoracic rudiment are the imaginal rudiments of the meso-thoracic legs and sternal region (*v. ms.*) ; the distal stalks of this pair of imaginal discs are attached to the body-wall at the posterior border of the fourth segment. The dorsal meso-thoracic imaginal discs, from which originate the mesonotal region and the wings, may be termed the alar or wing discs. They form a pair of flattened pyriform saca (fig. 22, *d.ms.*), lying one on each side of the ventral side of the fifth segment and slightly external to the lateral tracheal trunk (fig. 28, *d.ms.*), to a ventral branch of which each is attached. The meta-thoracic discs consist of two pairs of small pyriform masses (fig. 22) lying immediately behind the alar discs in the intersegmental line. They are attached to a ventral branch of the lateral tracheal trunk. The anterior rudiment (*v. mt.*) is the larger, and forms the imaginal meta-thoracic leg and sternal region ; in the blow-fly and Volucella it is interesting to note that this pair of imaginal discs is situated further

forward, and is in association with the corresponding pro-thoracic and meso-thoracic ventral discs. The smaller and more posterior disc (*d. mt.*) will develop into the remaining portion of the much reduced meta-thoracic segment, including the halteres.

Reference has already been made to other imaginal rudiments which occur in the abdominal region as circular patches of embryonic cells. The abdominal segments develop from numerous segmentally arranged plates of a similar nature, which are found during the early pupal stage.

During pupation the imaginal rudiments increase in size and are not destroyed by the phagocytes in histolysis, as is the case with most of the larval structures. The cephalic discs are evaginated by the eversion of their sacs by way of the anterior end of the larva, a cord of cells attached to the dorsal wall of the anterior end of the pharynx marking the path of eversion. A similar process takes place in the case of the thoracic imaginal discs, which, by their eversion, build up the whole of the skeletal case of the thorax and its dorsal and ventral appendages, the wings, halteres and legs.

VI. Summary.

1. An account of the previous work on the breeding habits of M. domestica is given, which, together with the author's investigations, show that the house-fly breeds in the following substances:

Horse-manure; this is preferred by the female flies as a nidus for the eggs, and forms the chief substance in which they breed; human excrement, either in the form of isolated fæces or occurring in such places as latrines, privies and ash-pits; cow-dung; poultry excrement; also in substances contaminated or mixed with excremental products, such as bedding from piggeries and from rabbits and guinea-pigs, paper and textile fabrics which have been contaminated, as cotton and woollen garments, sacking, rotten flock-beds, straw-mattresses, cesspools; decaying vegetable substances,

such as vegetable refuse from kitchens and decaying grain; rotten fruit, as bananas, apricots, cherries, plums, peaches and melon-slices; in spent hops; in waste food-stuffs, as bread moistened with milk, boiled egg, broth; bad meat and dead animals.

2. The most important factor in the development is temperature; a high temperature accelerates the development. Others factors concerned in the development are—the nature of the food and moisture, the effects of which are shown. Fermentation is also an important factor in development, as first shown by de Geer.

3. The shortest time occupied in the development, that is, from the deposition of the egg to the exclusion of the imago, is eight days, which period is obtained when the larvæ are incubated at a constant temperature of about 35° C.; under unfavourable conditions the development may extend over several weeks.

4. There are three larval stages, and the shortest times obtained for the development of the different developmental stages is—egg, from deposition to hatching, eight hours; first larval instar, twenty hours; second larval instar, twenty-four hours; third larval instar, three days; pupal stage, three days.

5. House-flies usually breed from June to October, but if the necessary conditions of temperature and suitable food are present they are able to breed practically the whole year round; these conditions are not, as a rule, satisfied during the winter months, except in such places as warm stables, etc.

6. The flies become sexually mature in ten to fourteen days after their emergence from the pupa, and they may begin to deposit their eggs as early as the fourteenth day after emergence. Each fly lays from 120–150 eggs in a single batch, and it may lay as many as six batches during its life.

7. The anatomy of the adult larva is described in the second portion of the paper. The body of the larva is considered to be composed of thirteen segments, of which the remnant of the cephalic region or pseudo-cephalon forms the

first. The apparent single second segment is considered to be of a double nature.

8. The muscular system is described in detail. It consists of: (1) A segmentally-arranged series of flattened cutaneous muscles forming an almost perfect sheath below the hypodermis; (2) the muscles controlling the cephalo-pharyngeal sclerites and pharynx; (3) the cardiac and visceral muscles. The series of muscular actions which probably takes place during locomotion is described.

9. The central nervous system is concentrated to form a single compound ganglion in which eleven pairs of component ganglia can be recognised. On the dorsal side of the anterior end of the ganglion two cerebral lobes united in the median line above the œsophageal foramen are situated; these contain the rudiments of the optic and supra-œsophageal ganglionic structures of the fly. Eleven pairs of segmental nerves arise from the ganglion, and in addition to these three pairs of lateral nerves, and also a single pair and two median unpaired dorsal accessory nerves arise. The component ganglia are surrounded by a cortical layer containing large ganglion cells; the whole compound ganglion is enclosed in a capsular sheath.

The only sensory organs are two pairs of tubercles situated on the dorsal sides of the oral lobes. By their structure they indicate an optical function.

10. The alimentary tract is very long in the larva, the ventriculus being especially elongate. It consists of pharynx, œsophagus, proventriculus, ventriculus, intestine and rectum. In addition to a pair of salivary glands, whose ducts unite to open by a single duct at the anterior end of the pharynx, and a pair of bifurcating Malpighan tubes, the larva possesses four cæca at the anterior end of the ventriculus. The ventriculus and intestine are very convoluted and are coiled up to form a complicated visceral mass.

11. The tracheal system of the adult larva consists of two longitudinal lateral tracheal trunks united by anterior and posterior commissures, and communicating with an exterior

by means of an anterior and a posterior pair of spiracles. The anterior spiracles, which do not occur in the first larval instar, are considered to be functional.

12. The vascular system consists of: (1) A dorsal vessel, the posterior region of which is swollen to form a cardiac region or "heart" which communicates with a pericardial cavity by means of three pairs of lateral ostia; (2) the great ventral sinus, which forms the body cavity; and (3) the pericardial sinus. The pericardium is well supplied with tracheæ, which may assist in respiration, as in certain other insect larvæ. The adipose tissue cells which compose the large laminæ forming the fat-body are similar in structure to those of the fly.

13. Three groups of imaginal rudiments or discs can be recognised in the larva: (1) The cephalic discs, of which two appendicular pairs are situated at the anterior end of the larva and three pairs in front of the cerebral lobes of the ganglion; (2) the thoracic discs, two pairs of which are attached to the anterior end of the ventral side of the ganglion, and three pairs are connected with the lateral tracheal trunks in the fifth segment; (3) the abdominal and visceral discs.

VII. Literature.

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EXPLANATION OF PLATES 30—33,

Illustrating Mr. C. Gordon Hewitt's paper on "The Structure, Development, and Bionomics of the House-fly Musca domestica, Linn. Part II. The Breeding Habits Development and the Anatomy of the Larva."

PLATE 30.

Fig. 1.—Eggs of M. domestica, × 40, dorsal and dorso-lateral views.
a. Anterior end.

Fig. 2.—Egg immediately before emergence of the larva which can be seen through the dorsal split of the chorion through which it emerges.

Fig. 3.—Posterior end of mature larva (3rd instar).
an. Anus. *p.sp.* Posterior spiracle.

Fig. 4.—Cephalopharyngeal skeleton of mature larva, left lateral aspect.
d.p.s. Dorsal pharyngeal sclerite. *d.s.* Dentate sclerite, *h.s.* Hypostomal sclerite. *l.p.* Lateral pharyngeal sclerite or plate, deeply incised posteriorly to form dorsal and ventral processes. *m.s.* Mandibular sclerite.

Fig. 5.—Mature larva of M. domestica.
a.sp. Anterior spiracular process. *an.l.* Anal lobe. *sp.* Spiniferous pad. I–XIII. Body segments.

Fig. 6.—Ventral aspect of the Pseudocephalon and second body segment of the mature larva showing the two oral lobes traversed by the food channels.
l. Lingual-like process. *m.* Mouth. *m.s.* Mandibular sclerite. *o.t.* Anterior optic tubercle.

Fig. 7.—Transverse section through two of the papillæ of the anterior spiracular process to show the clear central lumen.
c.p. The cuticular processes.

Fig. 8.—Larva shortly after hatching (1st instar).
m.s. Mandibular sclerite. *p.sp.* Posterior spiracle raised on short tubercle. *sp.* Spiniferous pad.

Fig. 9.—Lateral (left) aspect of the anterior end of the mature larva.
I–IV. Body segments. *a.sp.* Anterior spiracular process showing seven spiracular papillæ. *m.s.* Mandibular sclerite. *o.t.* Optic tubercle. *ps.* Pseudocephalon.

Fig. 10.—"Nymph" of M. domestica dissected out of pupal case about 30 hours after pupation.

an. Swellings of nymphal sheath marking bases of antennæ. *cx.* Coxa of leg. *lb.* Labial portion of proboscis sheath. *lbr.* Labral portion of same. *n.sp.* Spiracular process of nymph. *w.* Wing in nymphal alar sheath.

Fig. 11.—Head of "nymph" (about 48 hours after pupation). Enclosed in nymphal sheath. To show the development of the imaginal proboscis.

an. Antenna. *c.e.* Compound eye. *fac.* Facialia. *lab.* Labrum. *mx.p.* Maxillary palp. *o.l.* Oral lobe.

Fig. 12.—Posterior end of larva in the second stage (2nd instar).

an. Anus. *p.sp.* Posterior spiracle.

Fig. 13.—Cephalopharyngeal skeleton of the first larval instar; the outline of the pharyngeal mass is shown in dotted lines.

t.s. T-shaped sclerite of the left oral lobe. Other lettering as in Fig. 4.

Fig. 14.—Longitudinal section through the surface of one of the oral lobes of mature larva to show the food-channels.

ch. Food-channel. *ct.* Outer layer of cuticular integument. *ct'.* Inner layer of the same. *hy.* Hypodermis.

Fig. 15.—Pupal case of M. domestica from which the imago has emerged, thus lifting off the anterior end or "cap" of the pupa; ventro-lateral aspect.

a.sp. Remains of the anterior spiracular process of larva. *l.tr.* Remains of the larval lateral tracheal trunk. *n.sp.* Temporary spiracular process of nymph. *p.sp.* Remains of the posterior spiracles of larva.

PLATE 31.

Fig. 16.—Muscular system of the body-wall of the right side. The straight dorsal line is the median dorsal line of the body, and the curved ventral line is the median ventral line.

I—XIII. Body segments. *an.l.* Anal lobe. *an.m.* Anal muscle. *c.r.* Cephalic retractor muscle. *d.v.* Dorso-ventral muscle of the terminal segment. *ex.d.l.* External dorso-lateral oblique recti muscles. *i.l.o.* Internal lateral oblique muscle. *in.d.l.* Internal dorso-lateral oblique recti muscles. *l.i.m.* Lateral intersegmental muscle. *l.m.* Lateral muscles. *l.tr.* Branch of lateral tracheal trunk communicating with the anterior spiracular process. *l.v.l.* Longitudinal ventro-lateral muscles. *p.sp.* Posterior spiracle. *s.d.* Stomal dilators. *v.c.r.*, *v'.c.r.* Ventral cephalic retractor muscles. *v.l.o.* Ventro-lateral oblique muscle. *v.o.* Ventral oblique muscle.

Fig. 17.—Oblique section through the pharyngeal mass of the larva in the direction and at the level shown by the line *a.b.* in Fig. 19. (Camera lucida drawing.)

e.o.m. Elongate oblique pharyngeal muscle. *l.p.* Lateral pharyngeal

sclerite. *m.* Accommodating membrane. *m.d.* Mandibular depressor muscle. *o.ph.* Oblique pharyngeal muscle. *ph.* Pharynx. *s.d.m.* Semicircular dorsal pharyngeal muscles. *tr.* Trachea. *v.c.p.* Ventral cephalic protractor muscle.

FIG. 18.—Oblique section through the pharyngeal mass of the larva at the level shown by the line *x.y.* in Fig. 19. (Camera lucida drawing.)

p.s. Pharyngeal sinus. *r.ph.* Roof of pharynx. *T.r.* T-ribs of the floor of pharynx. Other lettering as in Figs. 17 and 19.

FIG. 19.—Muscles of the cephalo-pharyngeal sclerites of the mature larva seen from the left side. The muscles of the body-wall have been omitted with the exception of the large cephalic retractor muscles.

a.b., *x.y.* Levels and direction of the oblique sections shown in Figs. 18 and 19. *c.r.* Cephalic ring. *d.c.p.* Dorsal cephalic protractor muscle. *d.m.* Right pharyngeal depressor muscle. *d.s.* Dentate sclerite. *f.p.* Chitinous floor of the posterior region of the pharynx showing the bases of the T-ribs. *h.s.* Hypostomal sclerite. *m.d.* Mandibular depressor muscle. *m.e.* Mandibular extensor muscle. *m.s.* Mandibular sclerite. *s.d.* Stomal dilator muscles. *sal.d.* Common salivary duct. *v.c.p.* Ventral cephalic protractor muscles. *v.c.r.* and *v'.c.r.* Ventral cephalic retractor muscles.

FIG. 20.—Visceral or stomatogastric nervous system of the mature larva. The position of the ganglion (*G.*) with the cerebral lobes (*c.l.*) is shown by means of the dotted outline.

c.g. Central visceral ganglion. *pv.g.* Proventricular or posterior ganglion.

FIG. 21.—Transverse section of one of the salivary glands of the mature larva. (Camera lucida drawing.)

FIG. 22.—Internal aspect of the posterior thoracic imaginal discs of the right side.

d.ms. Dorsal mesothoracic or alar imaginal disc. *d.mt.* Dorsal metathoracic imaginal disc. *l.tr.* Lateral tracheal trunk of the right side of larva. *v.mt.* Ventral metathoracic imaginal disc.

PLATE 32.

FIG. 23.—Nervous system of the mature larva. The dorsal accessory nerves are shown by single black lines, and the outline of the pharyngeal mass is indicated by the dotted line.

I—XIII. Body segments of the larva. *c.l.* Cerebral lobes. *m.c.d.* Major cephalic imaginal discs. *œ.* Œsophagus. *o.v.* Anterior (œsophageal branch) of visceral nervous system.

FIG. 24.—Left lateral aspect of the ganglion of the mature larva showing the origin of the nerves, position of the imaginal discs, and anterior end of the dorsal vessel.

1—11. Eleven segmental nerves. *a.b.* and *c.* Nerves arising from the bases

of the stalks of the prothoracic and ventral mesothoracic imaginal discs. *c.l.* Cerebral lobe. *c.r.* Problematical cellular structure (Weismann's "ring"). *d.a'.*, *d.a''.*, *d.a'''.* Dorsal accessory nerves. *d.v.* Dorsal vessel. *m.c.d.* Major cephalic imaginal discs. *œ.* Œsophagus. *pr.d.* Prothoracic imaginal disc. *t.* Fine tracheæ which arise in association with the segmental nerves, others arise with some of the more posterior nerves, but for the sake of clearness they are not included in the figure. *tr'.*, *tr''.* Tracheæ entering the ganglion. *v.m.s.* Ventral mesothoracic imaginal disc. *v.n.* Visceral nerve.

Fig. 25.—Longitudinal section of the proventriculus of the mature larva (Camera lucida drawing.)

c.c. Large cells forming the central hollow core of the proventriculus. *ch.i.* Chitinous intima of the œsophagus. *e.v.* Epithelial cells continuous with and similar in character to those of the ventriculus. *i.c.* Ring of imaginal cells. *œ.ep.* Œsophageal epithelial cells. *v.c.* Lumen of ventriculus.

Fig. 26.—The longitudinal lateral tracheal trunk of the left side seen laterodorsally showing the origin of the tracheal branches; small portions only of the right trunk are shown.

a.com. Anterior tracheal commissure. *a.sp.* Anterior spiracular process. *f.b.* Fat-body. *or.l.* Oral lobe. *l.tr.* Longitudinal lateral tracheal trunk. *p.com.* Posterior commissure. *p.sp.* Posterior spiracle. *tr'.* Trachea entering ganglion anteriorly. *tr''.* Trachea entering ganglion laterally. *v.tr.* Visceral tracheal trunk.

Fig. 27.—Longitudinal sections through the major cephalic imaginal discs of mature larva to show the position of the individual imaginal rudiments. The dextral section is more dorsal than the sinistral. (Camera lucida drawings.)

an.d. Imaginal disc of the antenna. *f.d.* Facial imaginal disc. *i.s.* Sheath of imaginal rudiments. *o.d.* Optic imaginal disc. *o.g.* Imaginal disc of the optic ganglionic structures. *o.s.* Optic stalks. *s.g.* Fundament of the imaginal supra-œsophageal ganglionic. *sh.* Sheath of cerebral lobe.

Fig. 28.—Transverse section of mature larva anterior to the ganglion and cerebral lobes to show the position of certain of the imaginal discs. The body-wall and muscles have been omitted. The folded character of the adipose tissue laminæ can be seen in this section, and also the degenerating anterior portions of the malpighian tubules (*m.t.*). (Camera lucida drawing.)

an.d. Antennal disc. *c.r.* Problematical cellular structure (Weismann's "ring"). *c.v.* Cæcum of ventriculus. *d.ms.* Dorsal mesothoracic (alar) imaginal disc. *f.c.* Adipose tissue cell. *l.tr.* Lateral tracheal trunk. *m.t.* Malpighian tubule cut rather longitudinally. *œ.* Œsophagus. *pr.d.* Prothoracic imaginal disc. *v.ms.* Ventral mesothoracic imaginal disc.

PLATE 33.

FIG. 29.—Alimentary system of mature larva. The course of the ventriculus and intestine as they lie in the larva is shown by the dotted lines. The origins only of the Malpighian tubes are shown.

c.s.d. Common salivary duct. *c.v.* Cæcum of ventriculus. *int.* Intestine. *m.t.* Malpighian tubule. *œ.* Œsophagus. *ph.* Pharynx. *pv.* Proventriculus. *r.* Rectum. *s.gl.* Salivary gland. *v.* Ventriculus.

FIG. 30.—Transverse section of a portion of the ventriculus of mature larva. (Camera lucida drawing.)

e.v. Epithelial cell of ventriculus showing large active nucleus and striated peripheral region of cell. *g.s.* Probable gland cells. *i.c.* Group of imaginal cells.

FIG. 31. Horizontal section of posterior or "cardiac" region of the dorsal vessel. (From camera lucida drawings.)

os. Ostium. *v.* Valvular flaps guarding the same.

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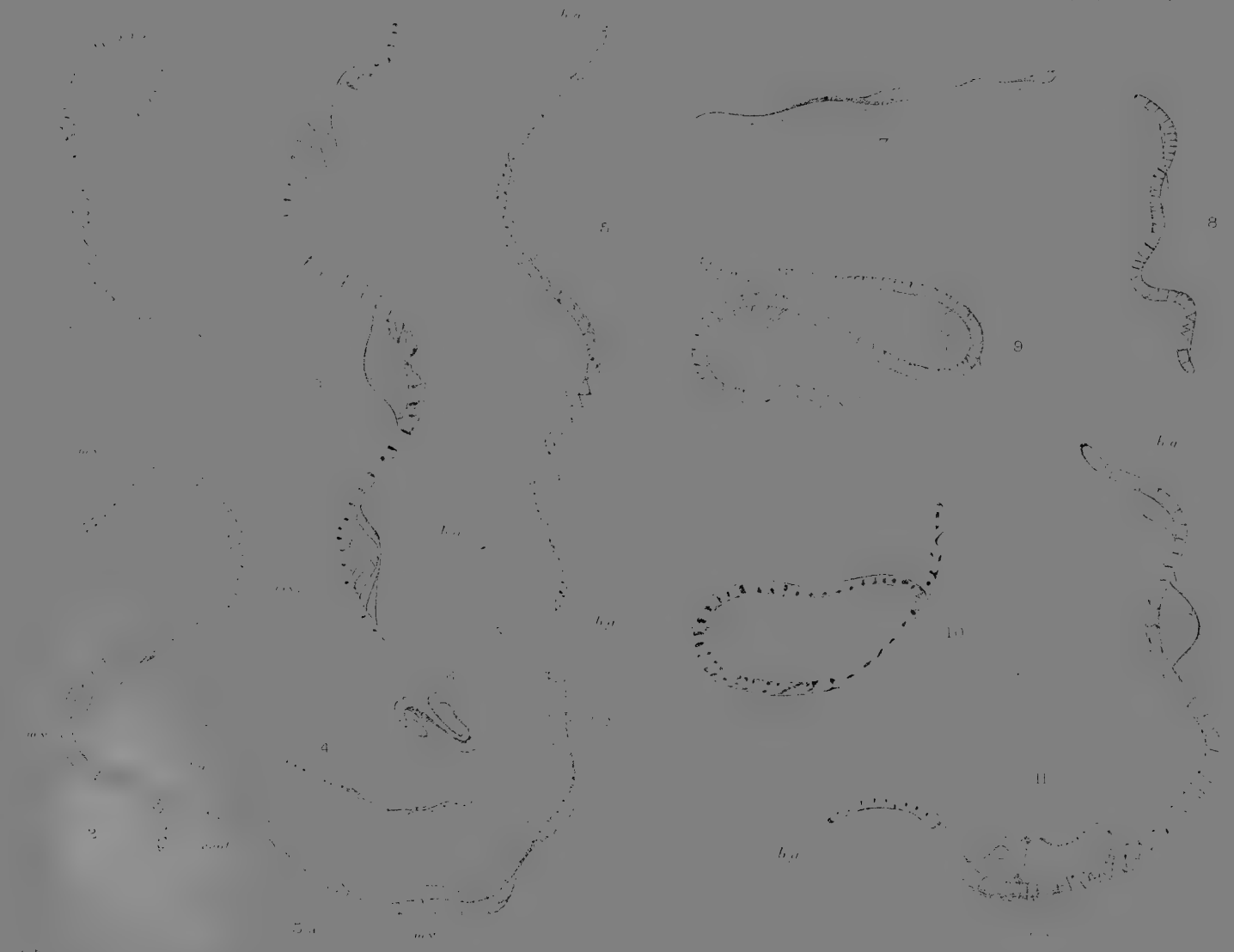

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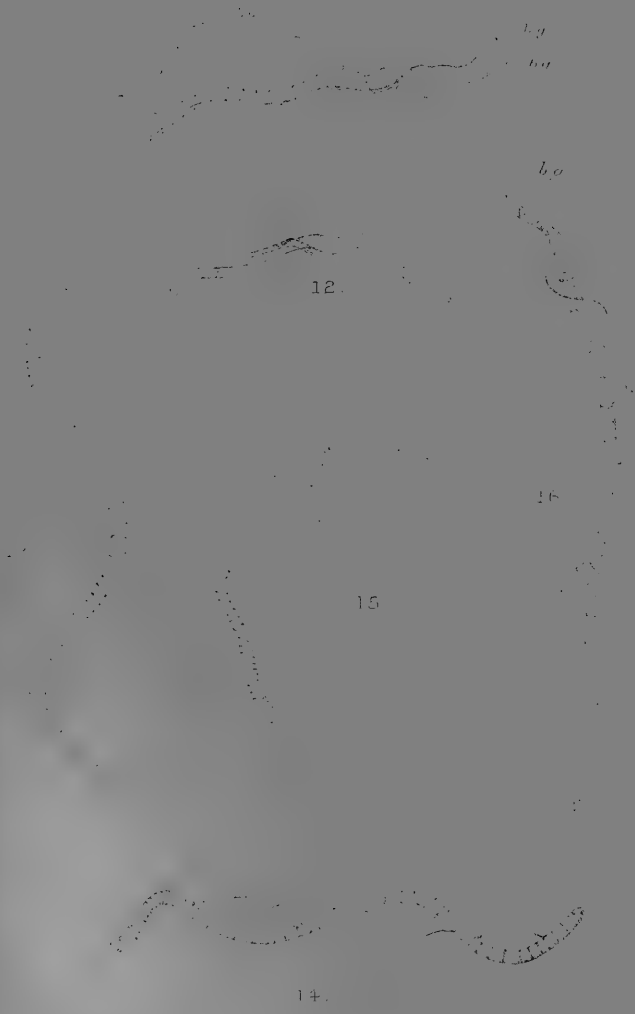

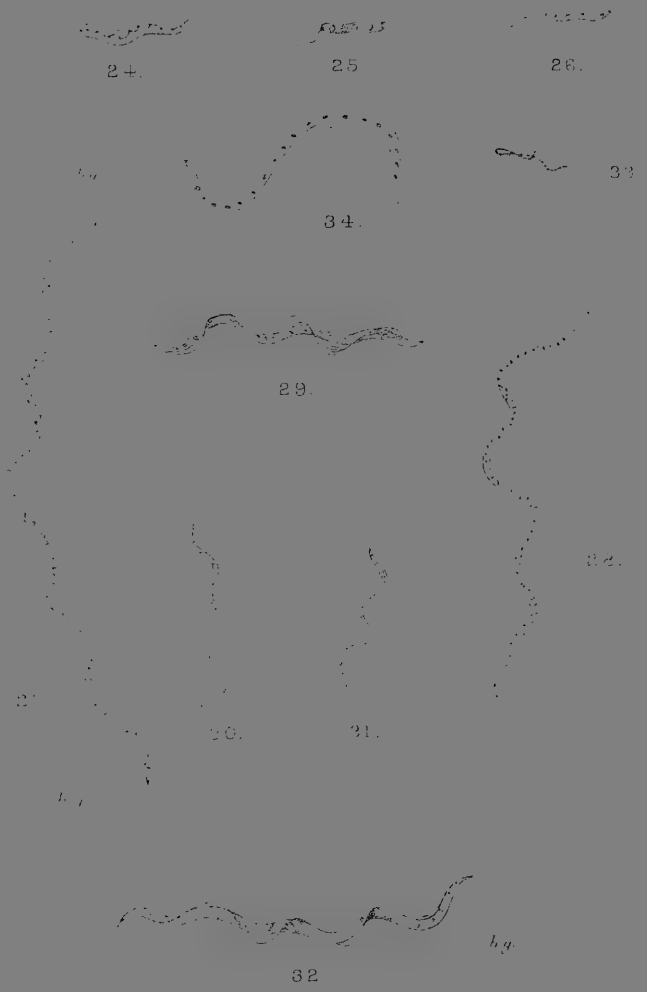

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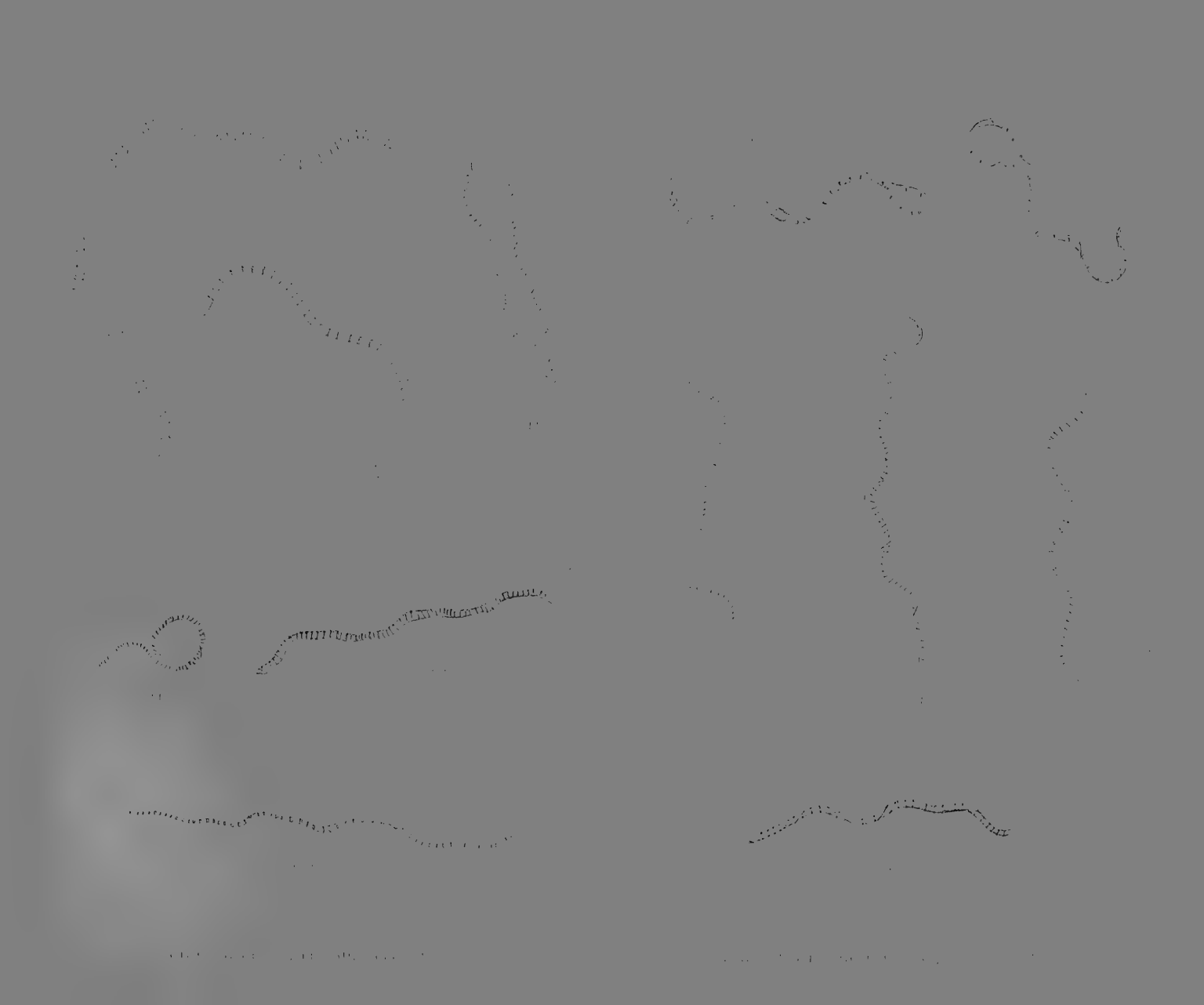

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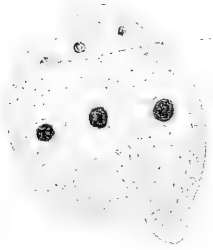

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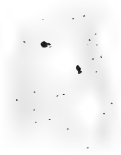

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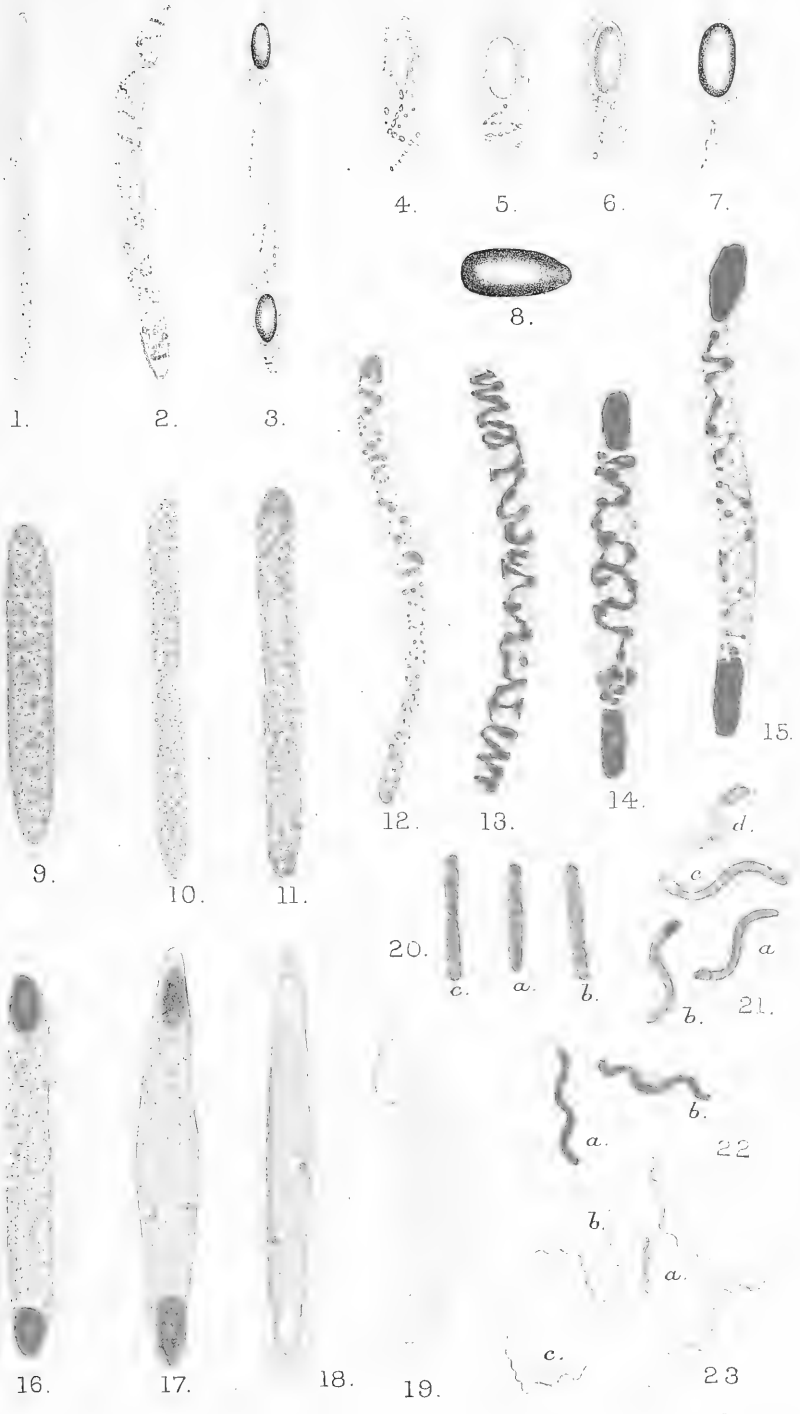

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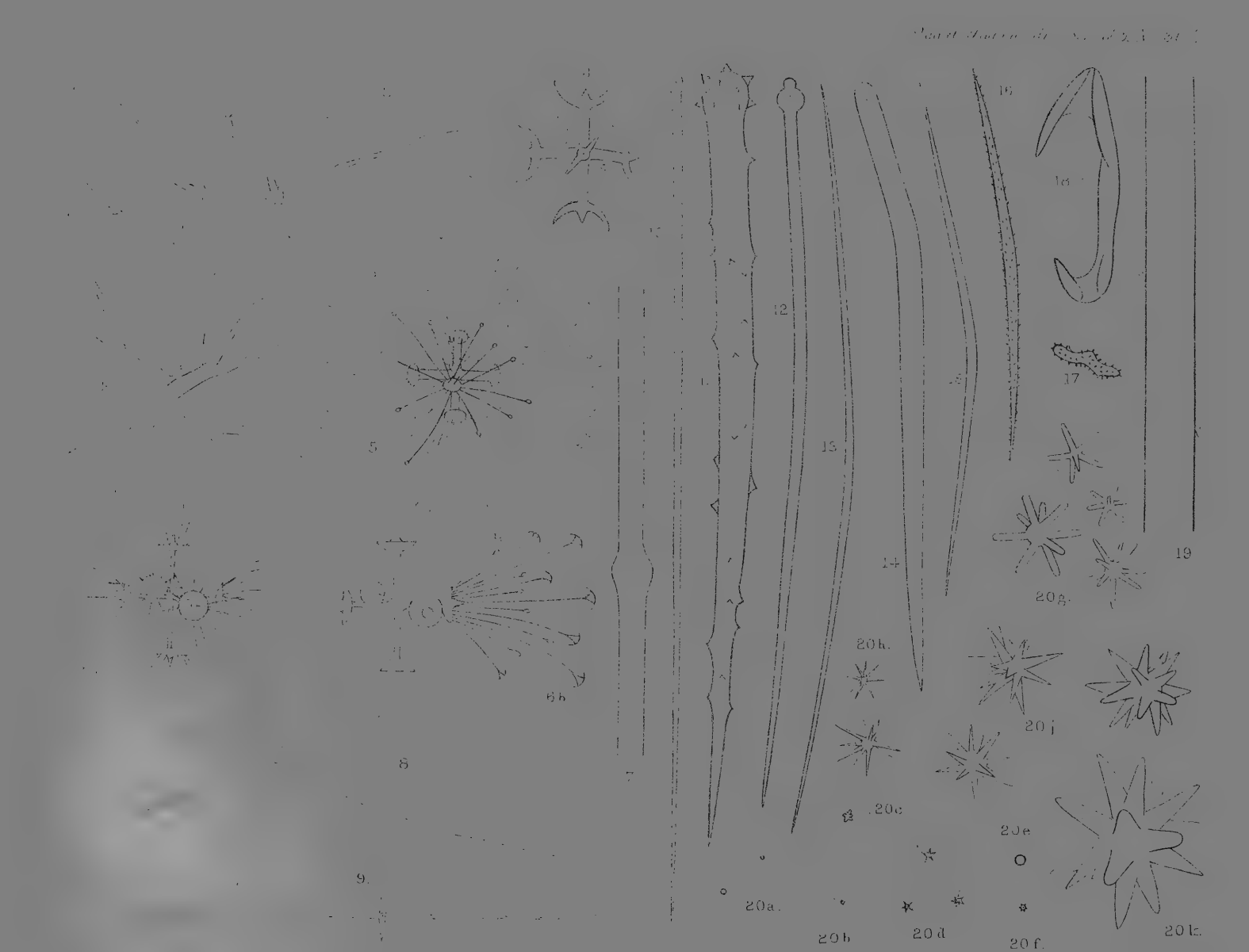

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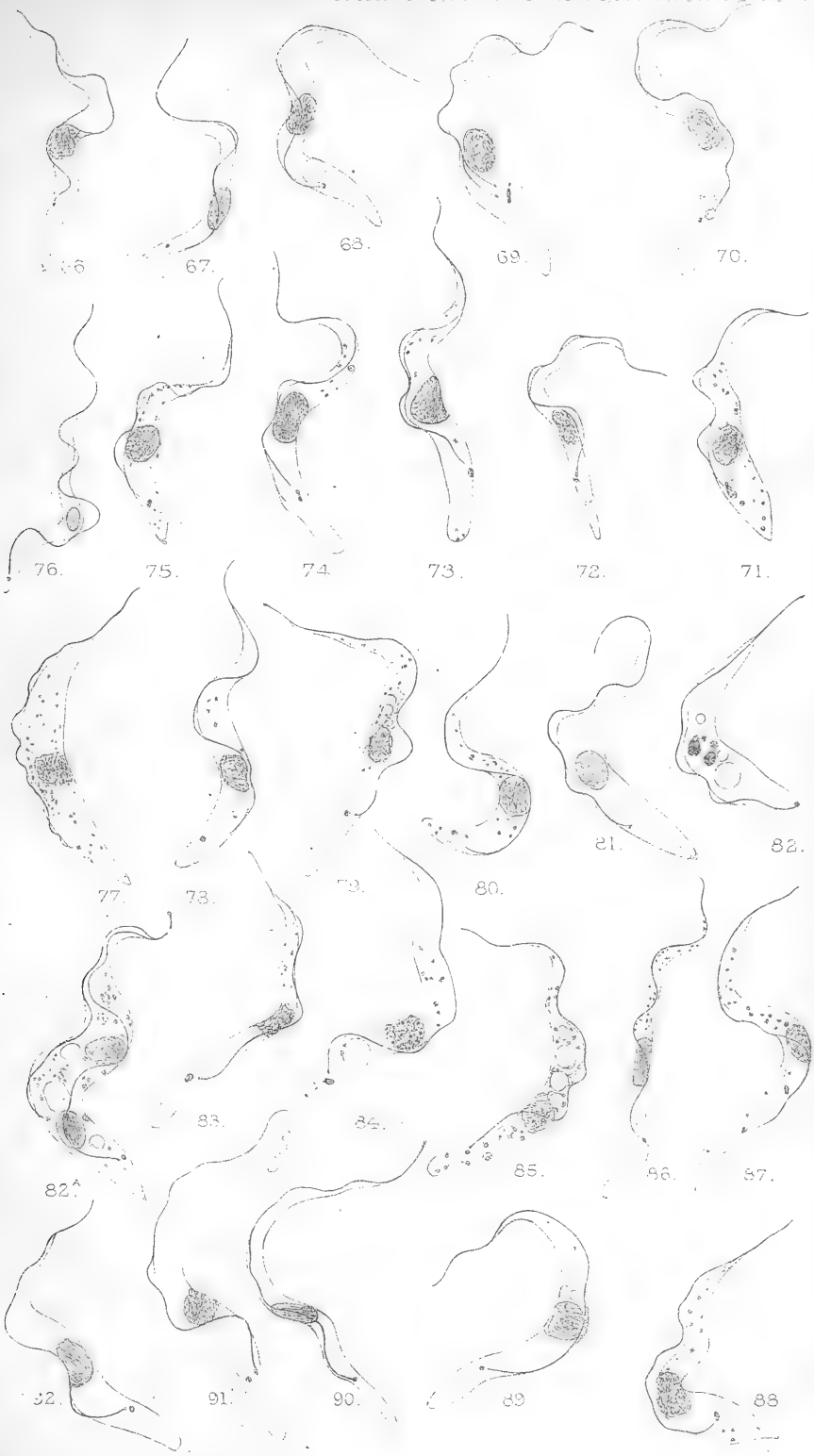

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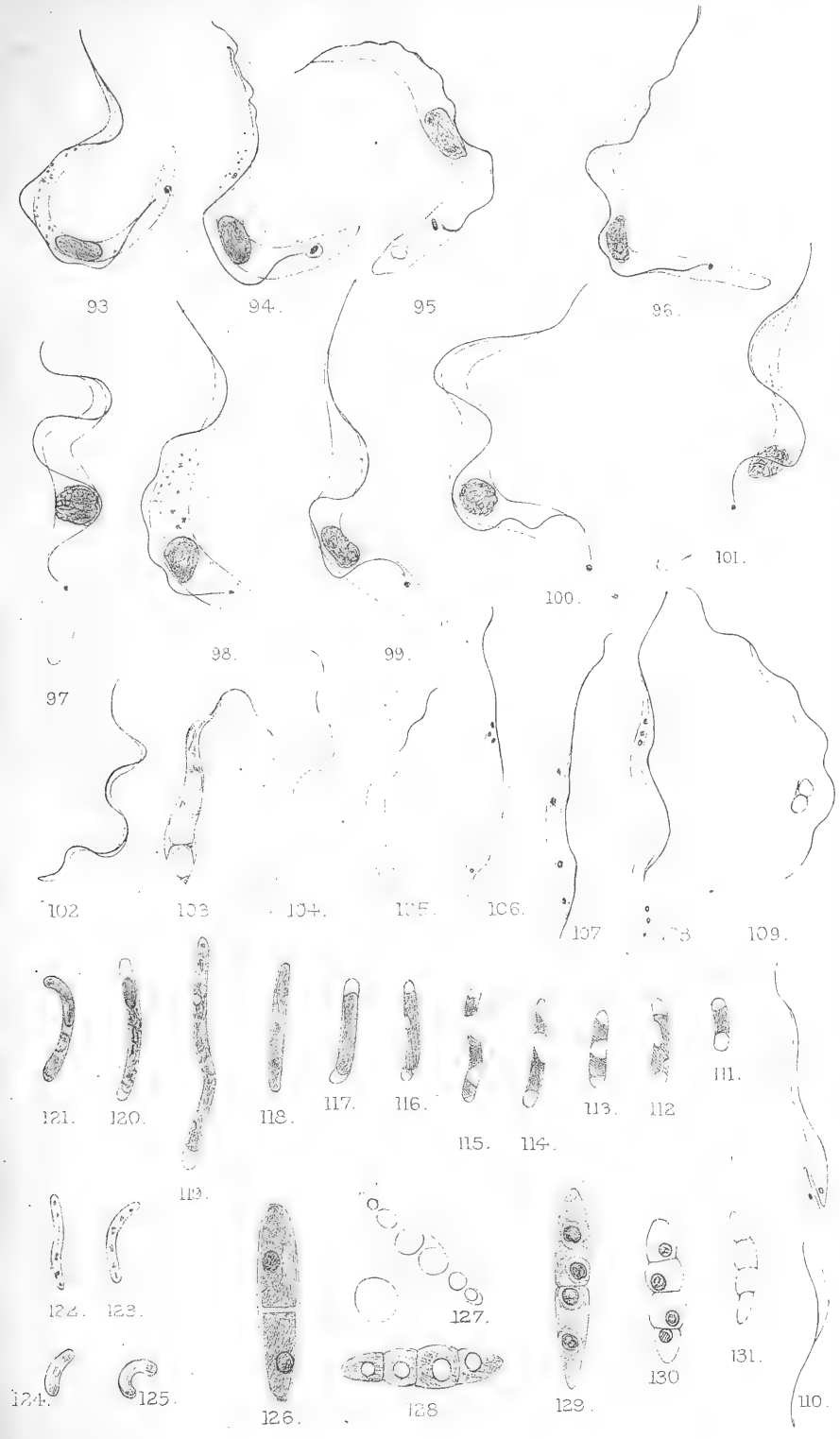

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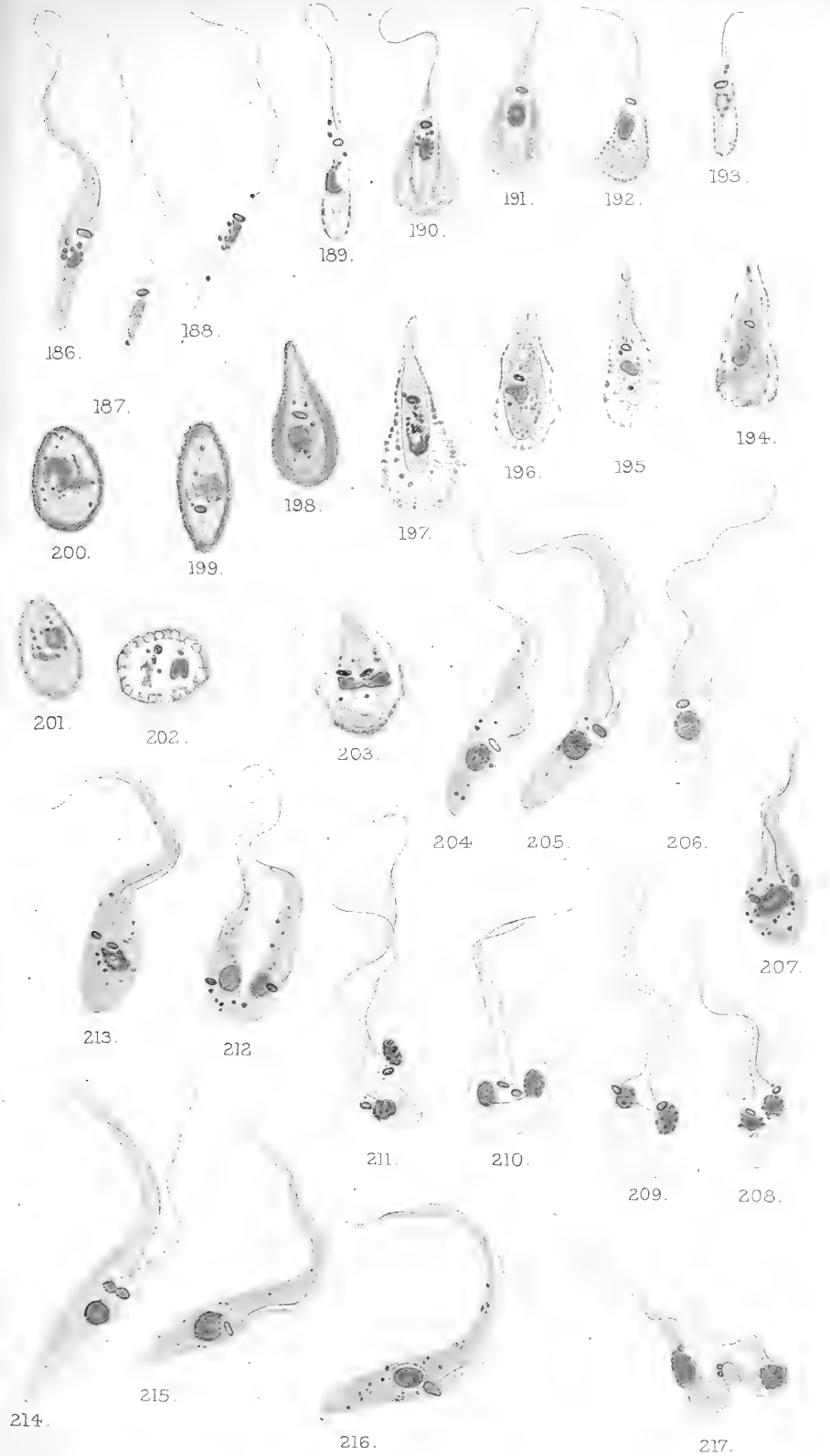
TRYPANOSOMA GAMBIENSE; (Figs. 93–110), BACTERIA, etc. (Figs. 111–131).

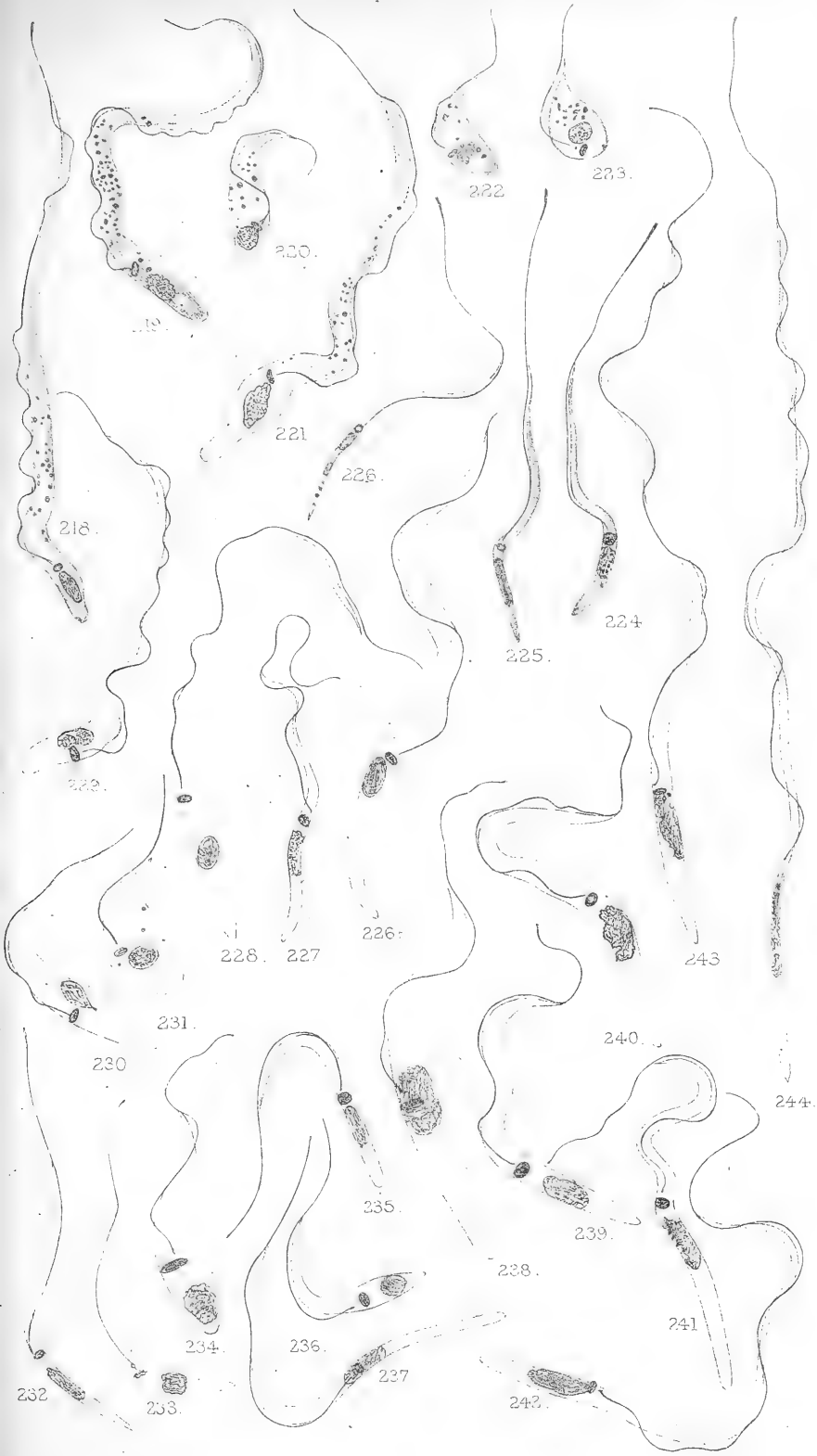
TRYPANOSOMA GRAY:



Ad. nat. del. E.A.M.

Huth. Lith. London.

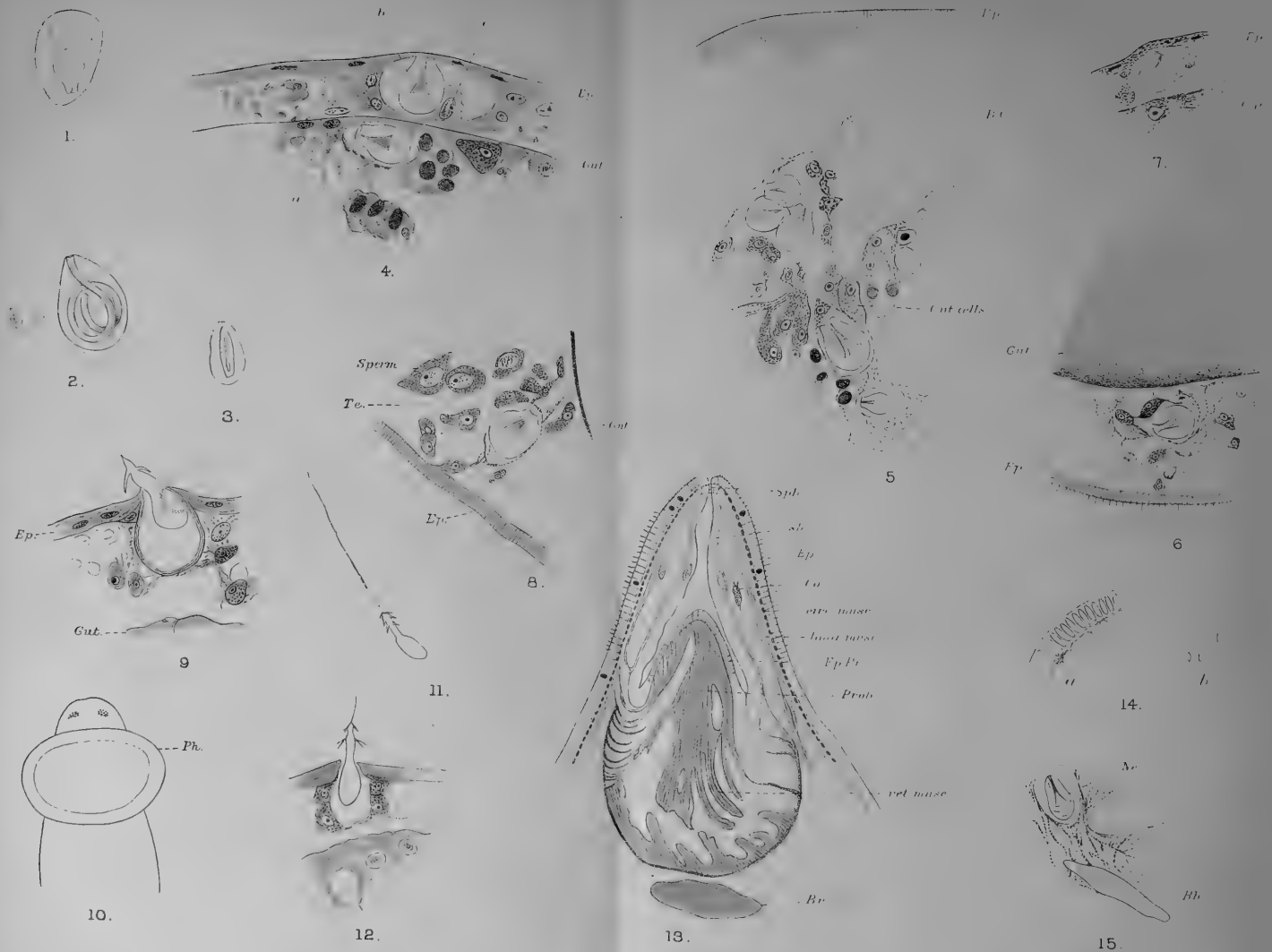
TRYPANOSOMA GRAYI (Figs. 186–216), T. GAMBIENSE (Fig. 217).



Ad. Nat. del. E.A.M.

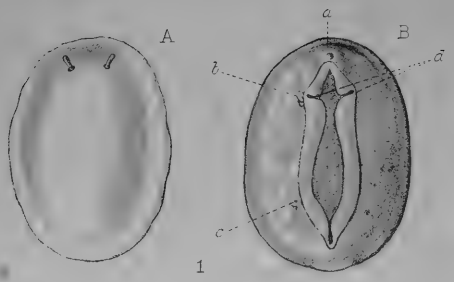
Huth. Lith^r London.

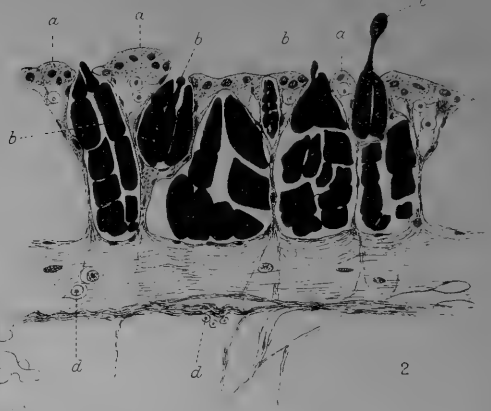
TRYPANOSOMA GRAYI.

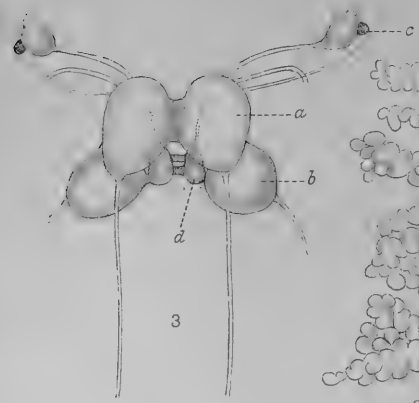


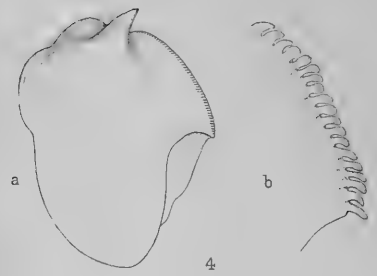
Martin and Wilson del.

NEMATOCYSTS OF TURBELLARIA.



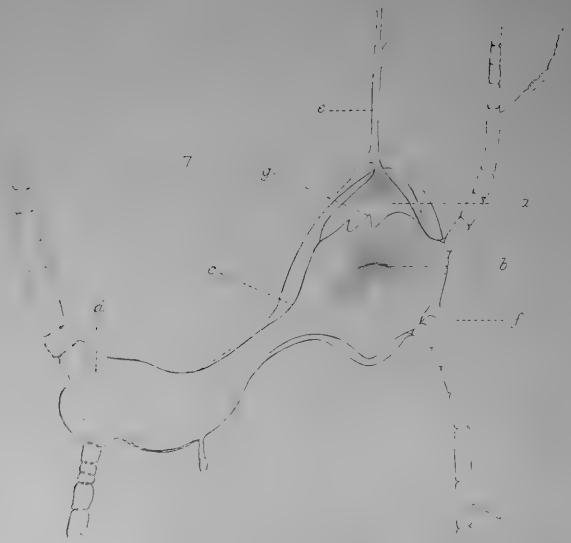


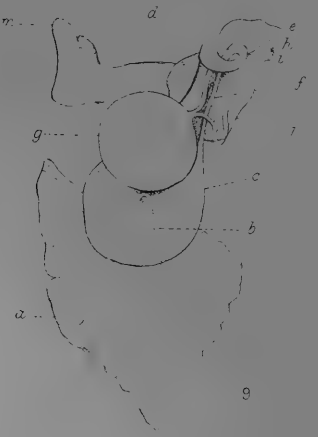


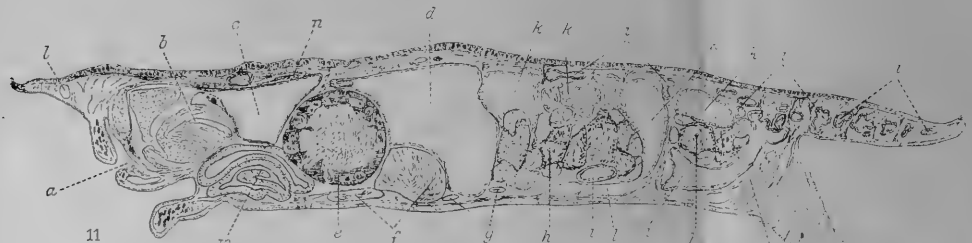


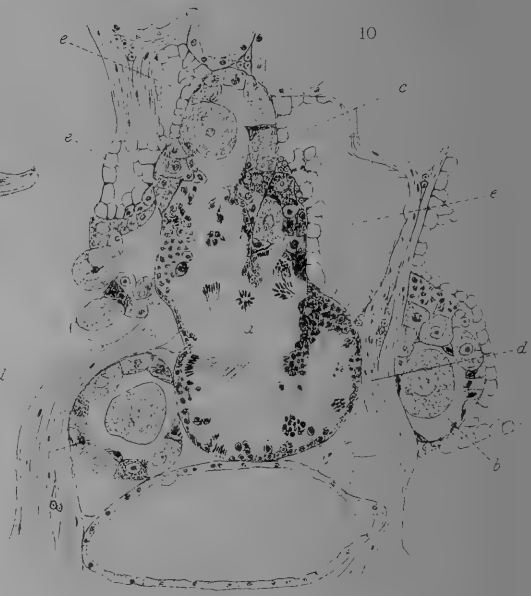
West, Newman lith.

DORIDOEIDES GARDINERI.









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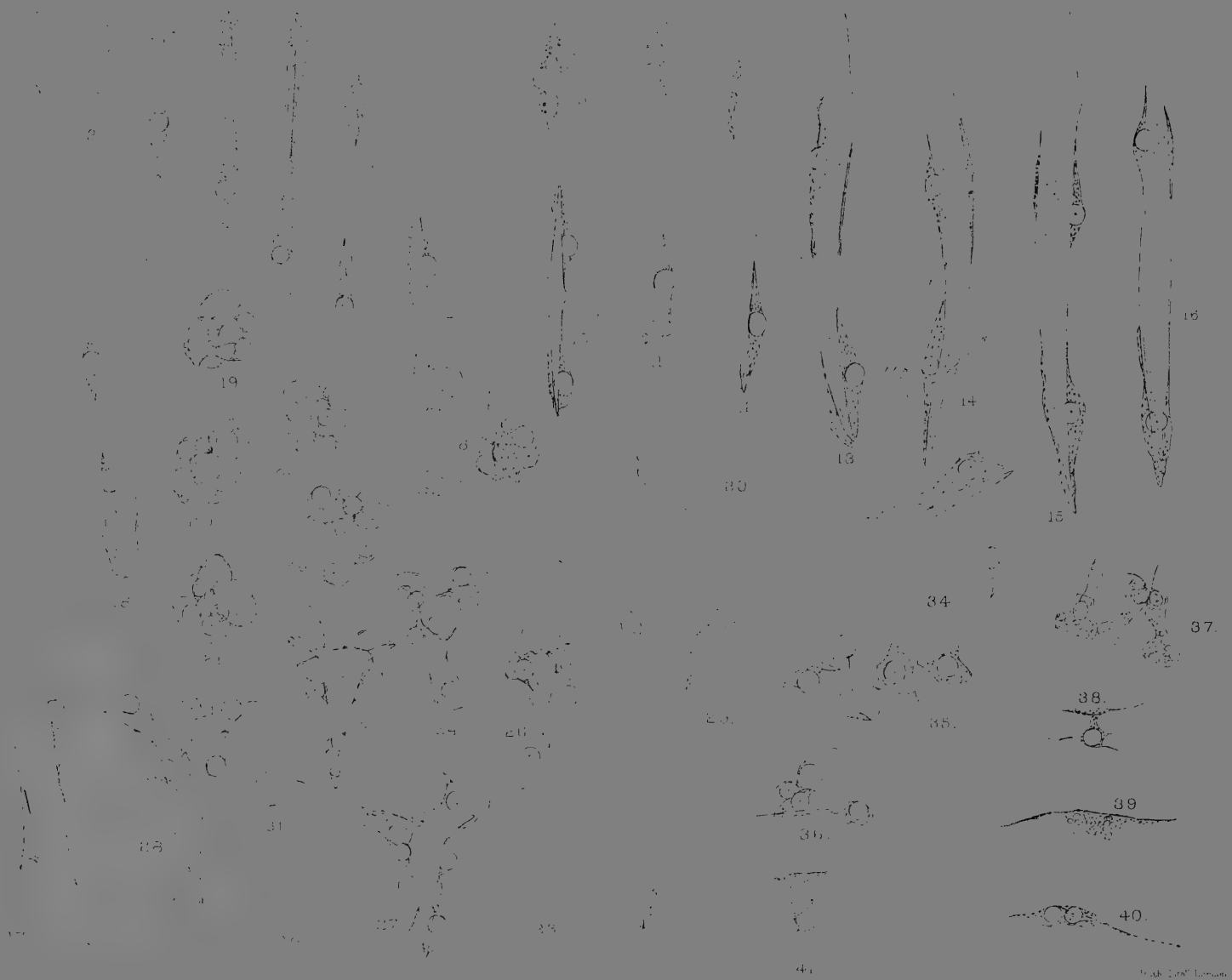
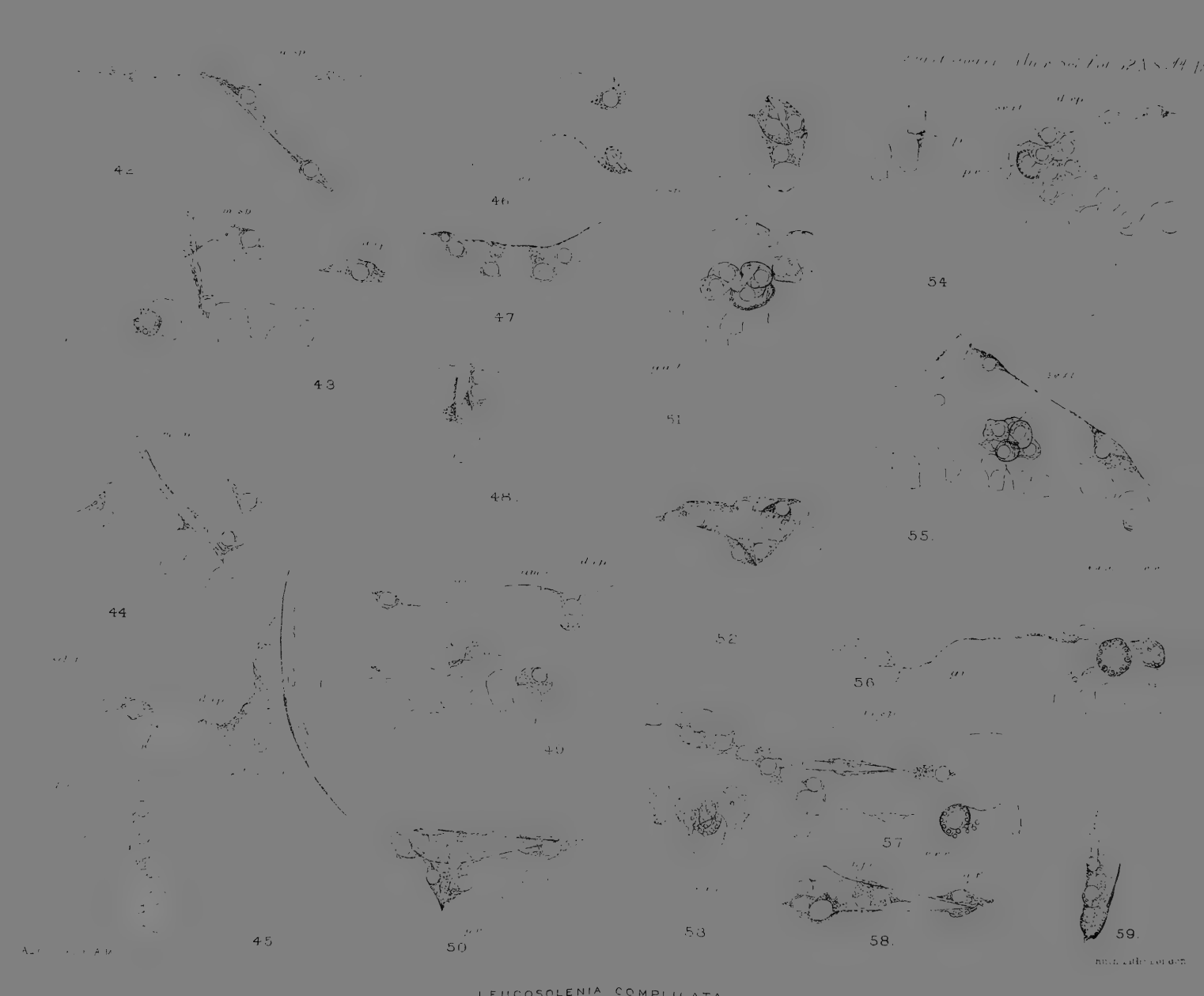
West, Newman lith.

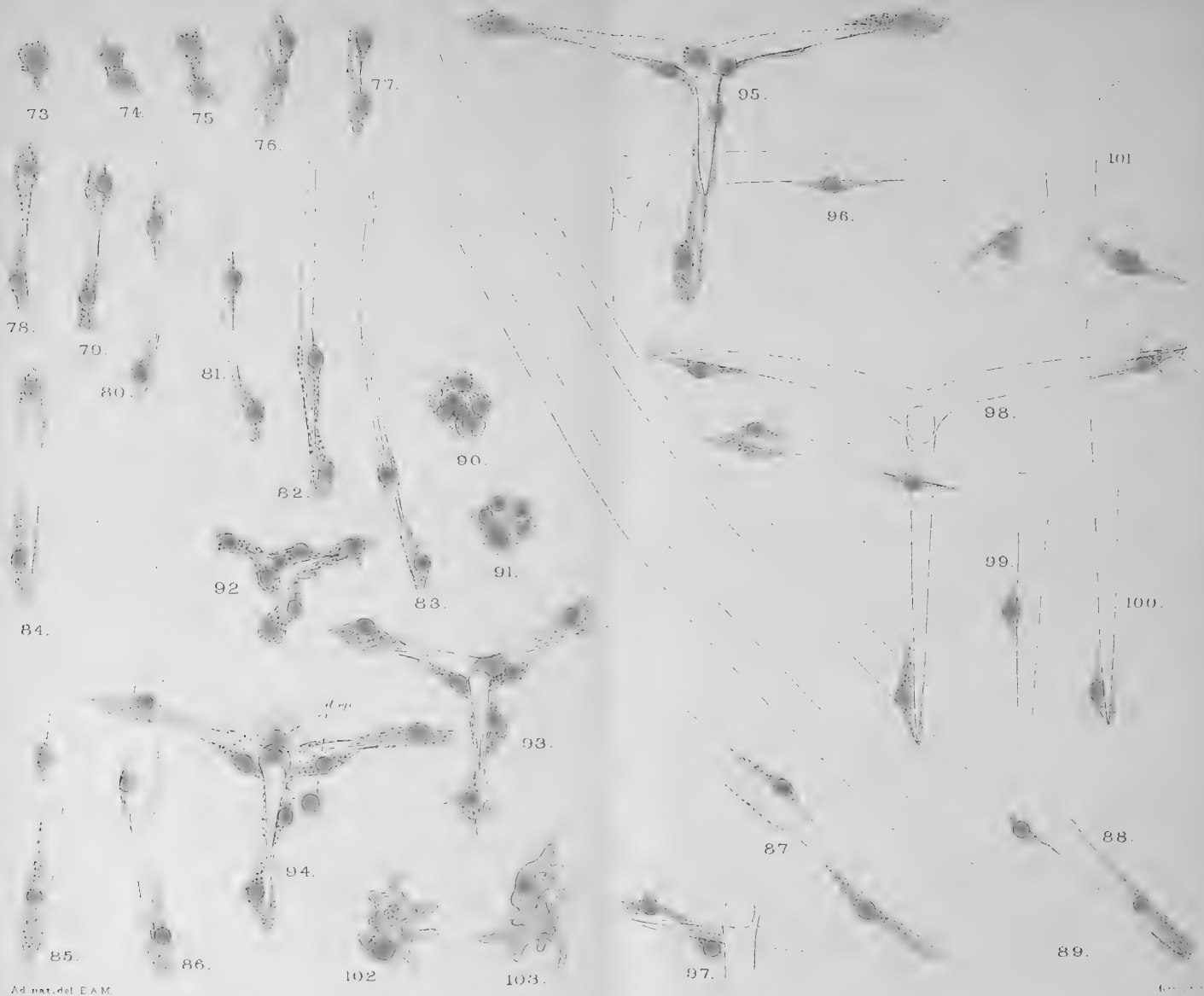


LEPTOSOLENIA SEMALICATA

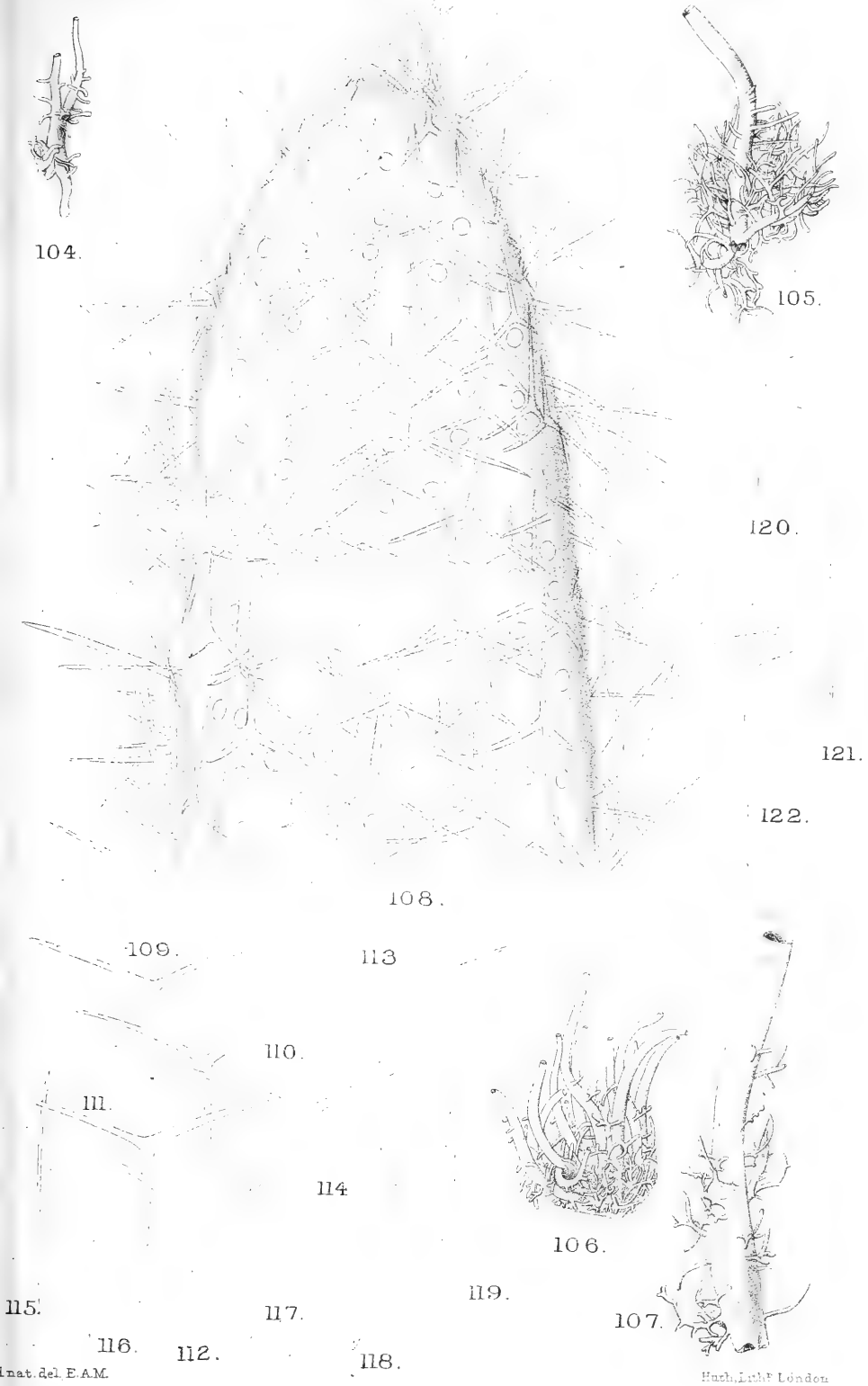


LEUCOSOLENIA COMPLICATA

LEUCOSOLENIA COMPLICATA



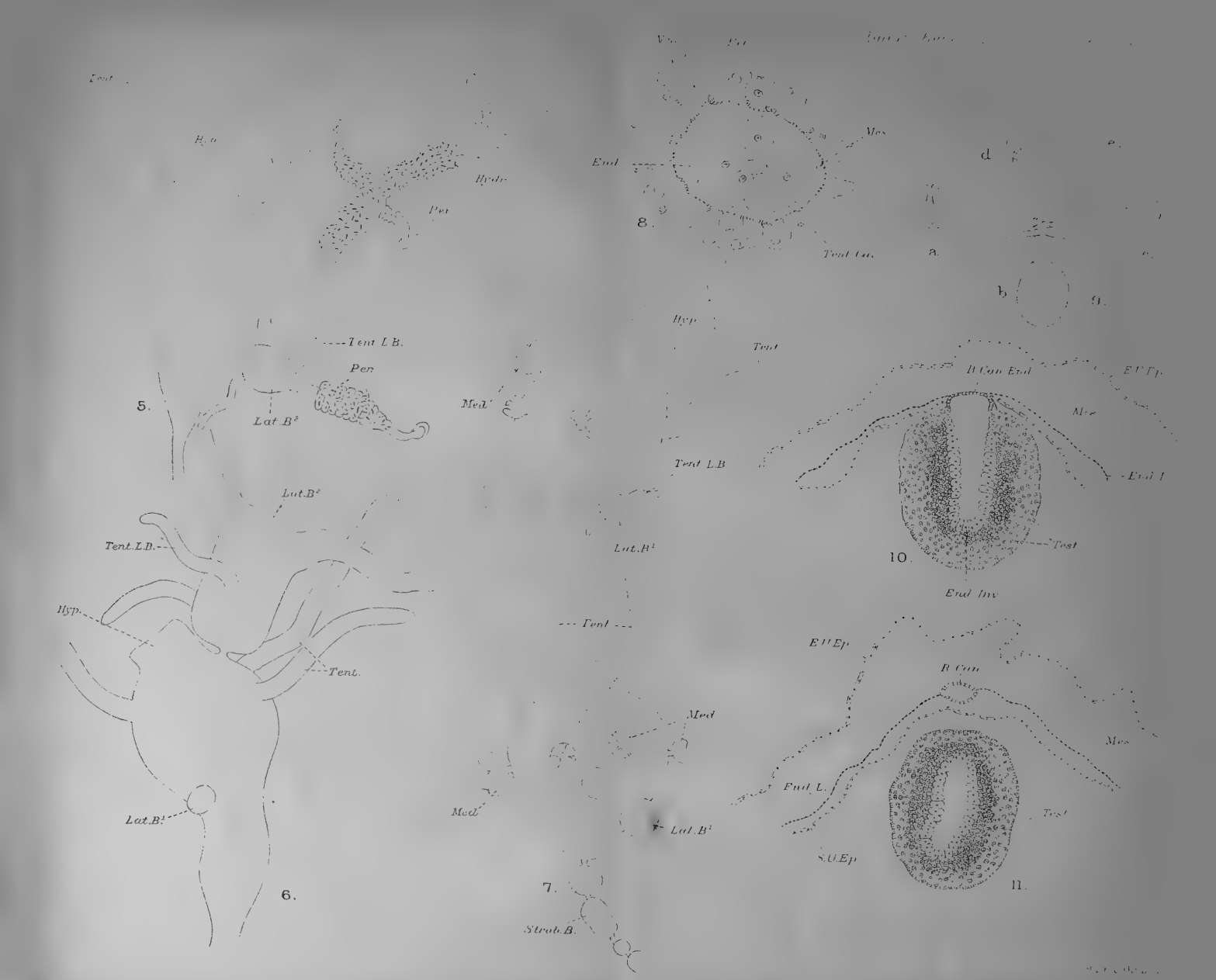
LEUCOSOLENIA VARIABILIS (73–100), CLATHRINA CORIACEA (101) and AMŒBÆ (102,103).



inat. del. E.A.M.

Huth, Lith^r London

LEUCOSOLENIA LIEBERKUEHNII.



MOERISIA LYONSI.

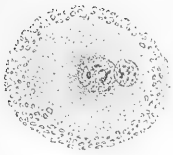


Fig. 2.

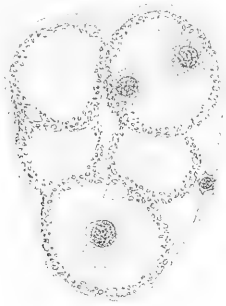
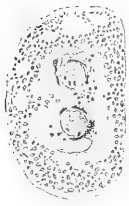


Fig. 3.



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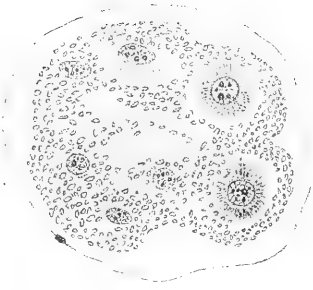

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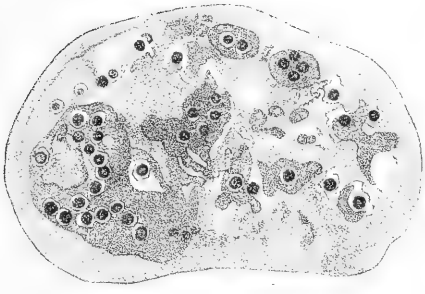

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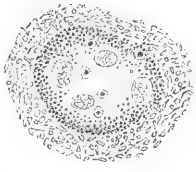

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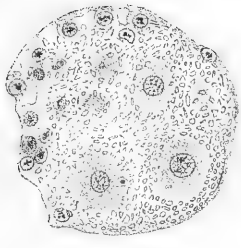

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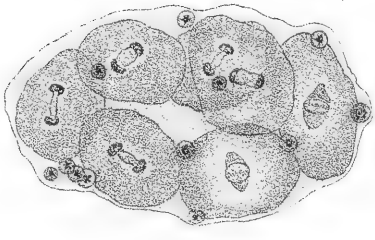
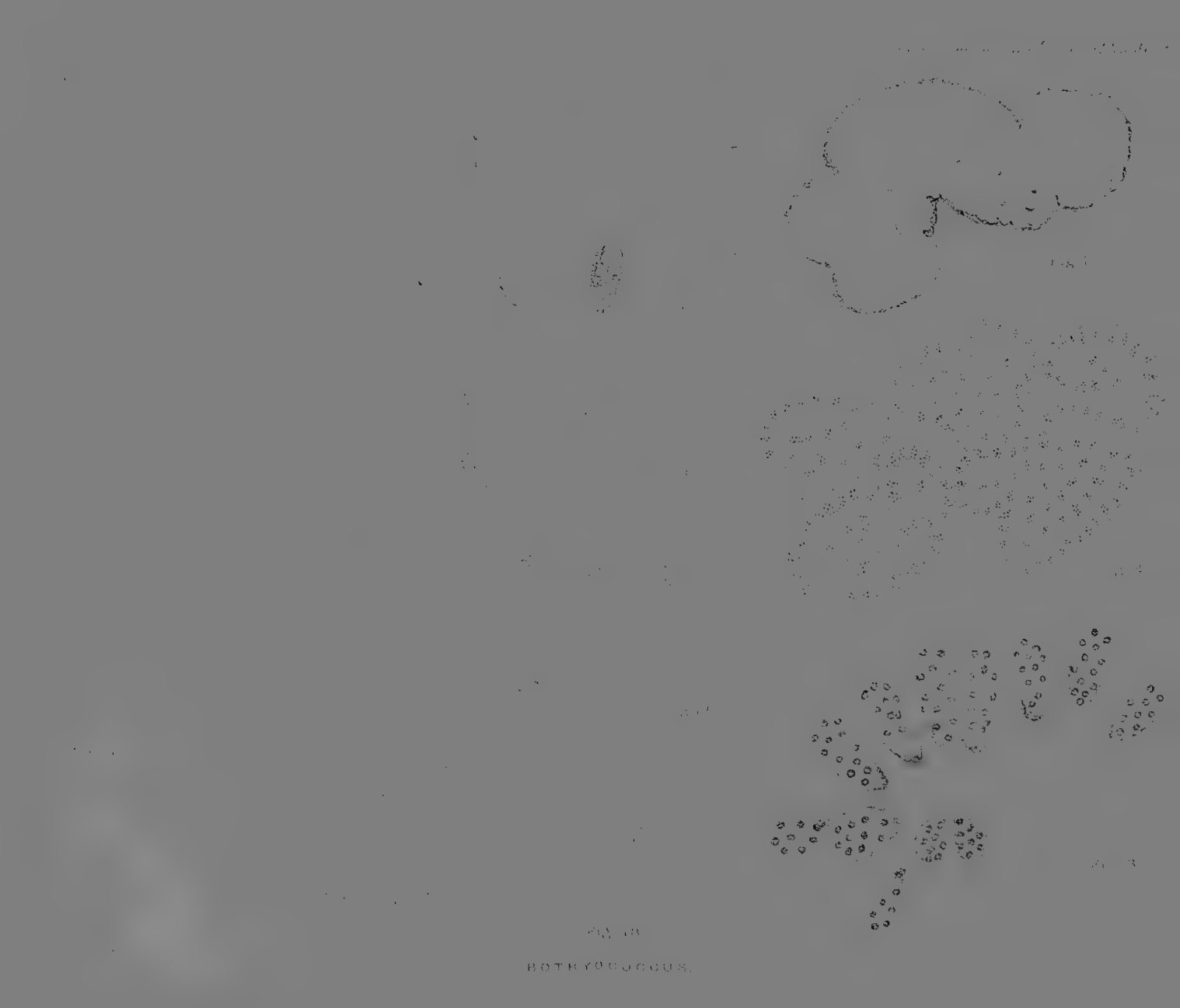


Fig. 9.

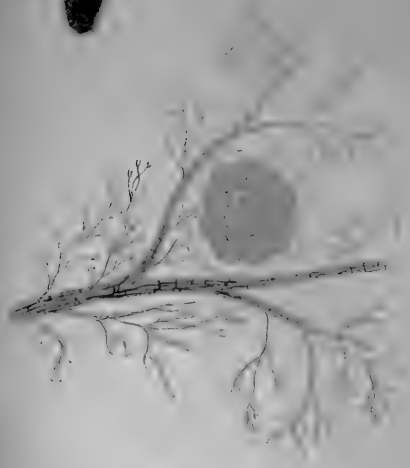
D.L. Mackinnon del.

Hick, Lith^r London.



BOTRYOCOCCUS.

Fig. 1.



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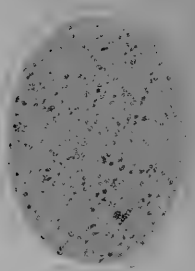


Fig. 3

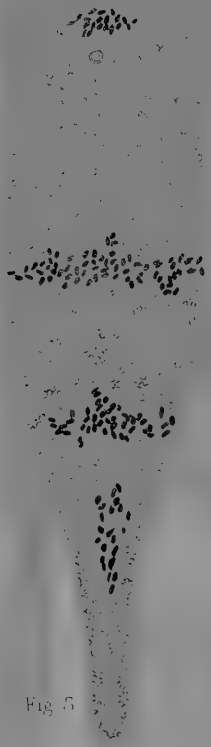


Fig. 5

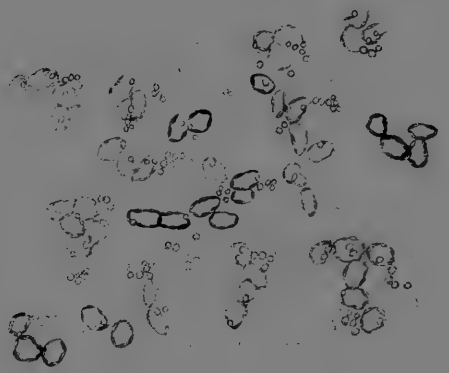


Fig. 6.

Fig. 4

F. Keeble del

CONVOLUTA PARADOXA

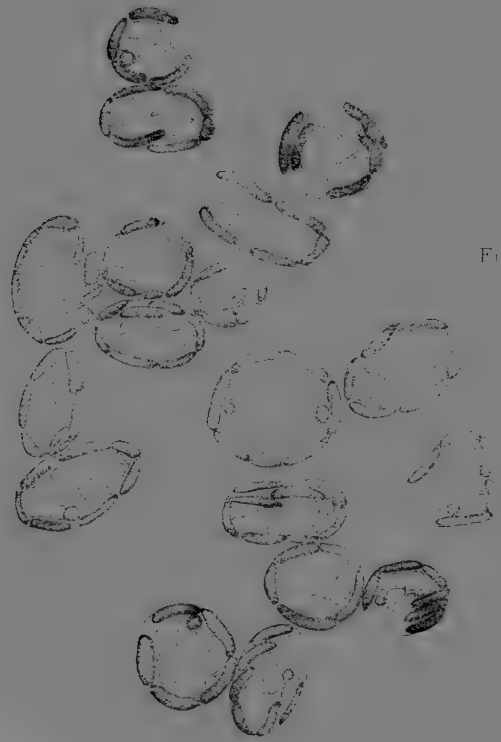


Fig 7

Fig 8

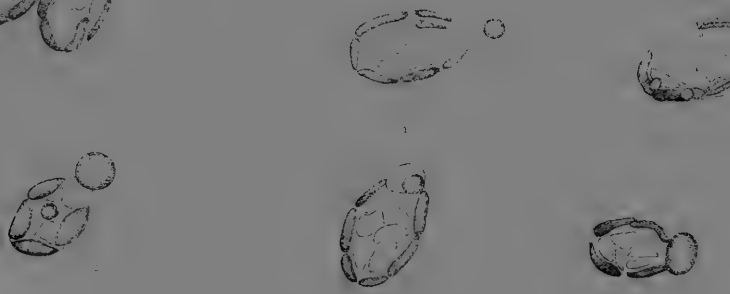


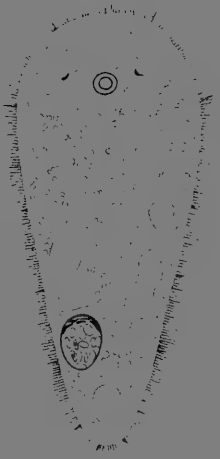
Fig 9.

Fig 10

Fig 11.

Fig 12

Fig 13



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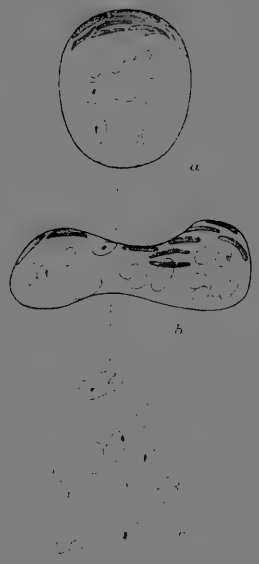

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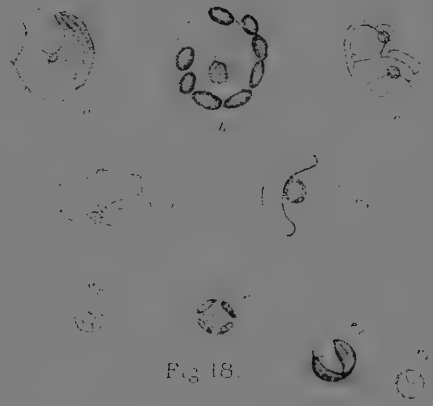
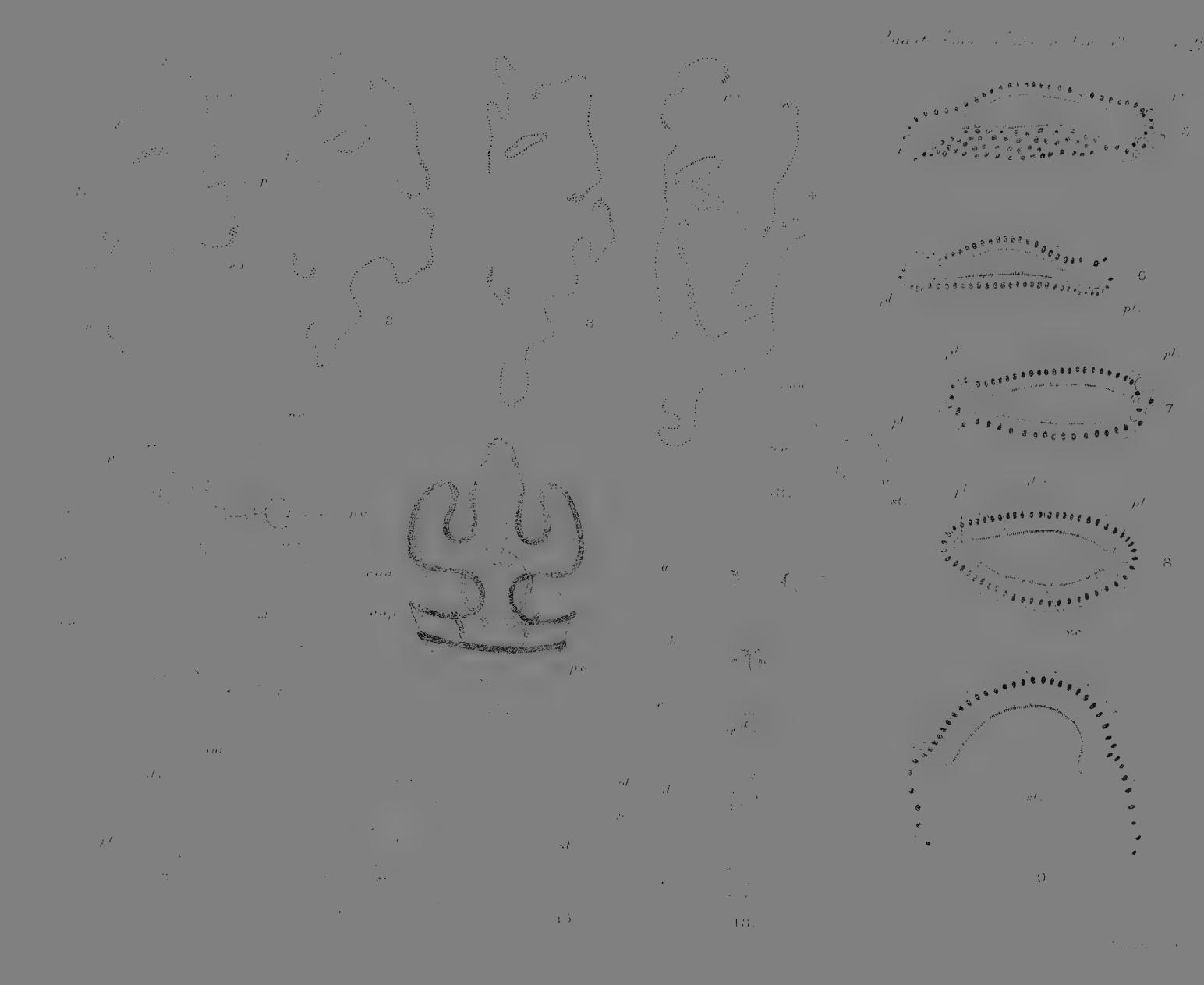


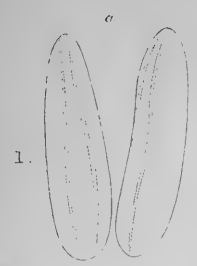
Fig 18.

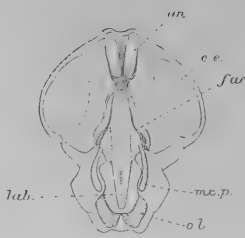
Fig 16.

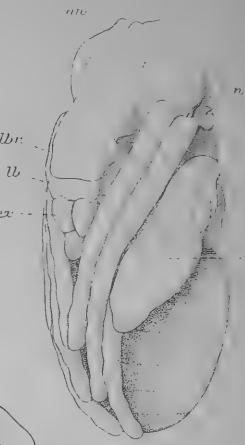
Fig 17.

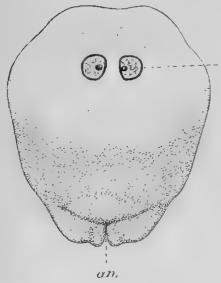
CONVOLUTA PARADOXA

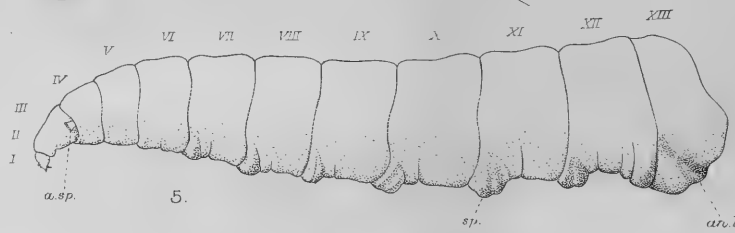


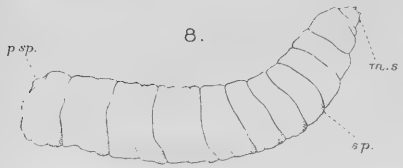


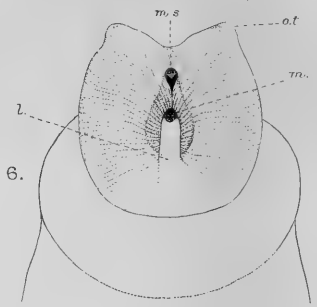


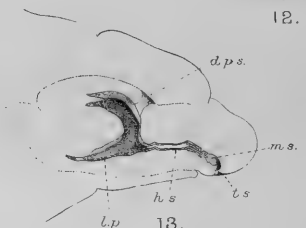


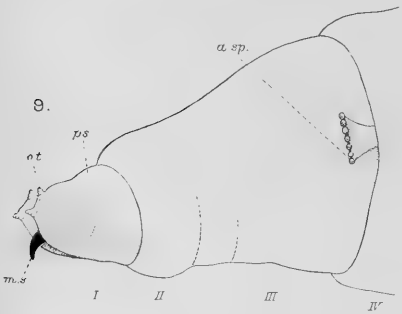




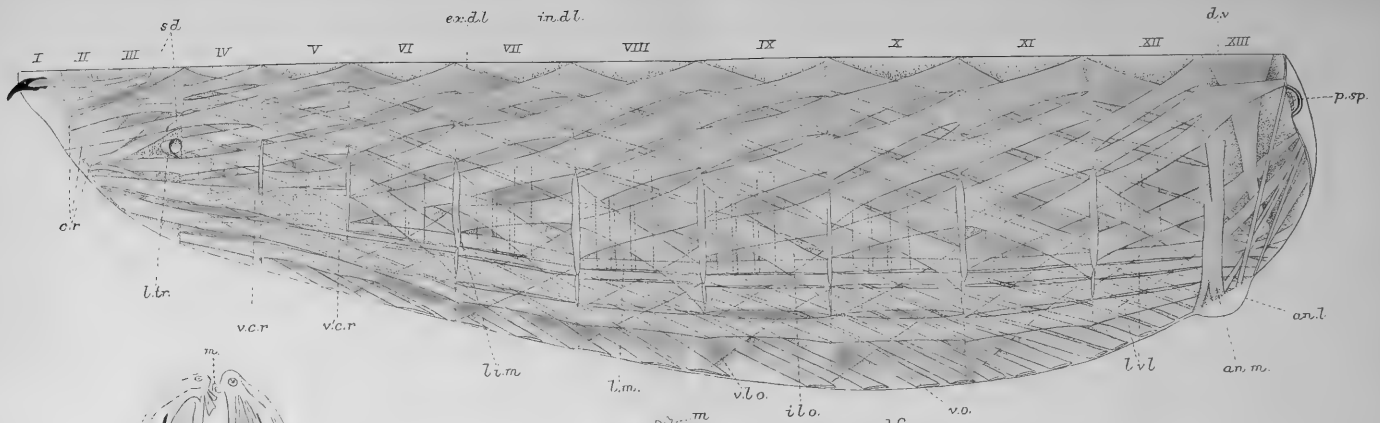








MUSCA DOMESTICA.



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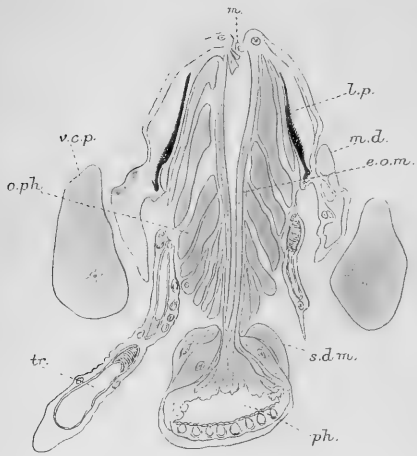

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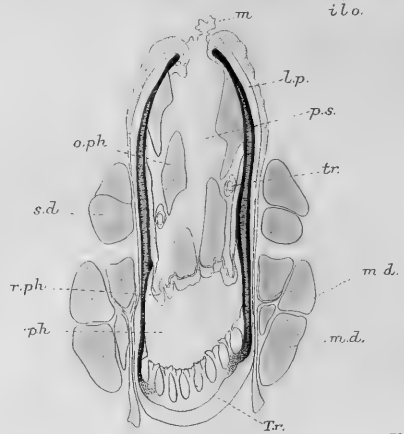

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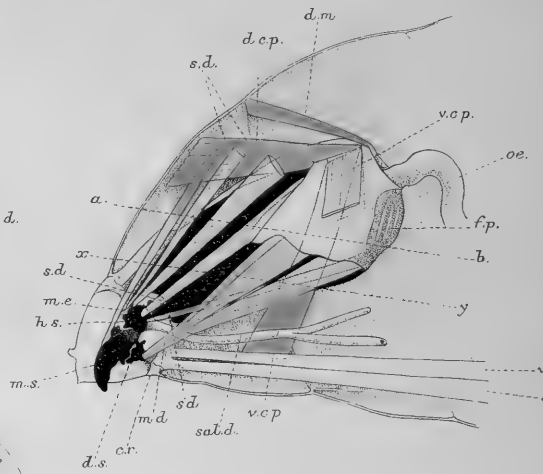

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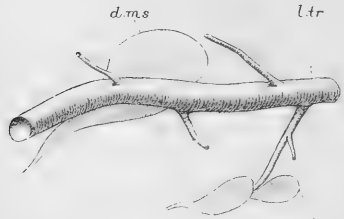

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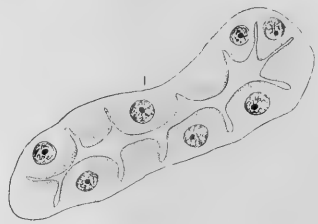

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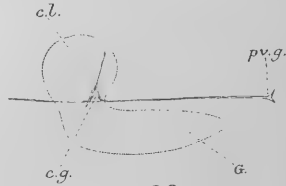
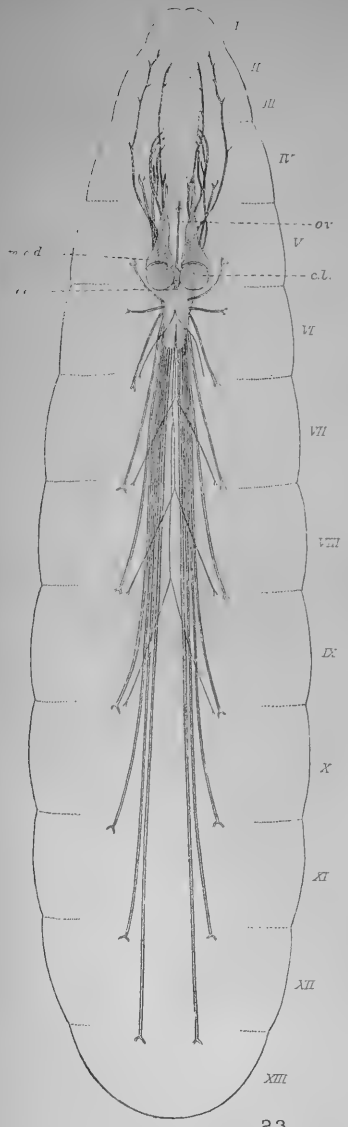


20.

Huth, Lith.r London

MUSCA DOMESTICA.



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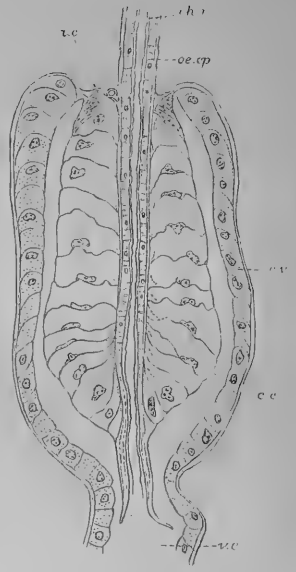

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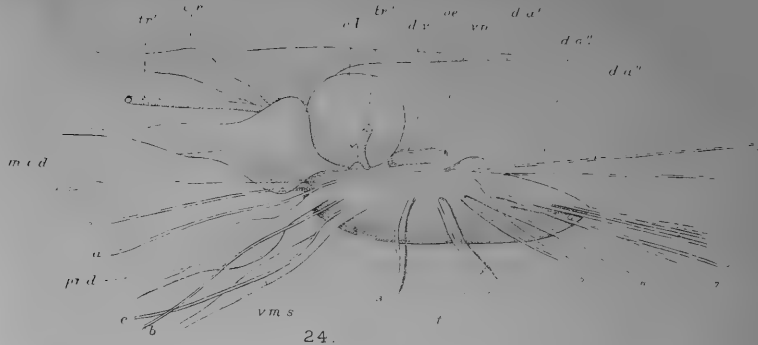
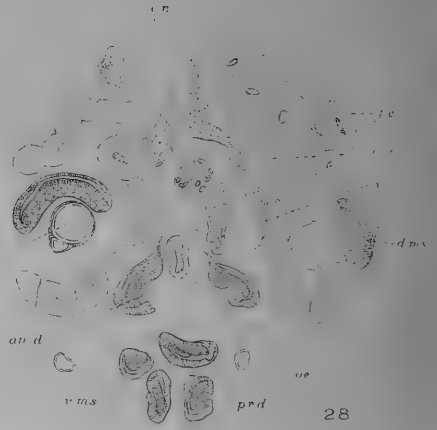
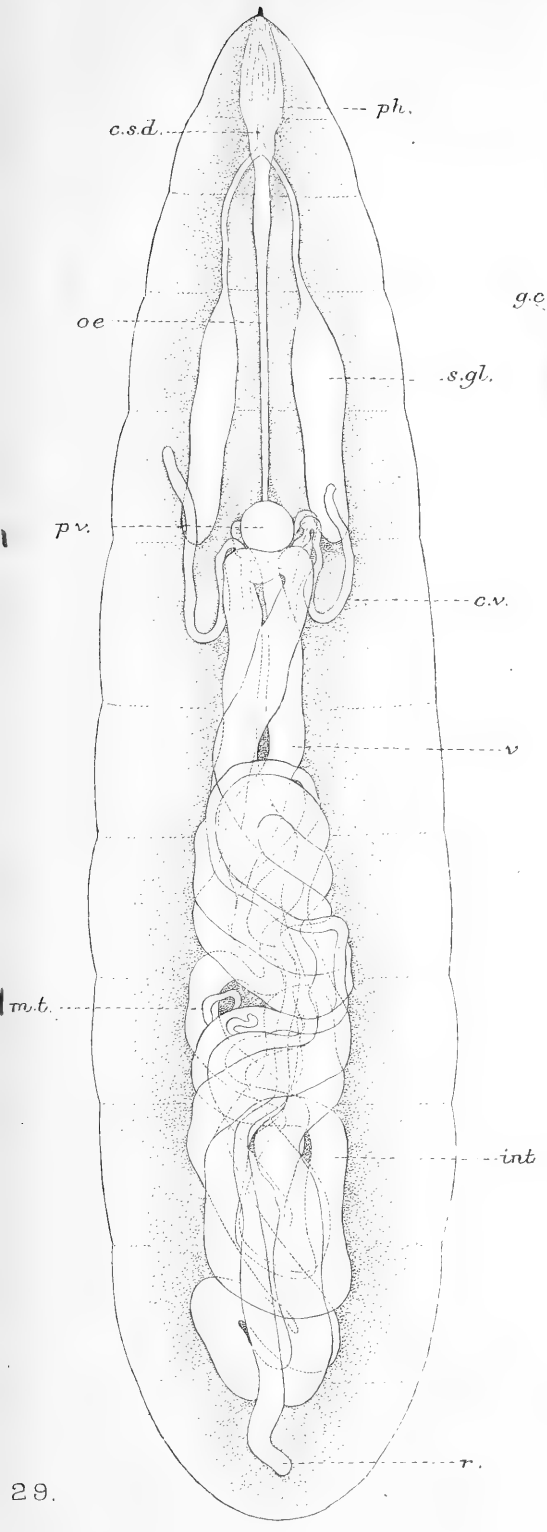




C.G.H. del.

MUSCA DOMESTICA.



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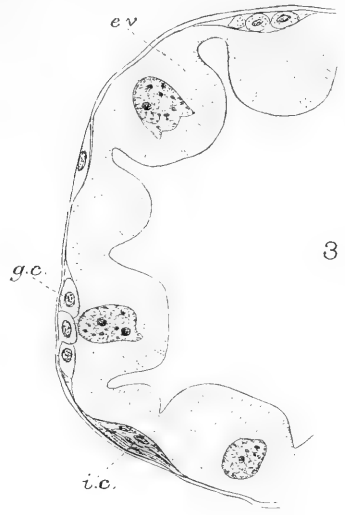

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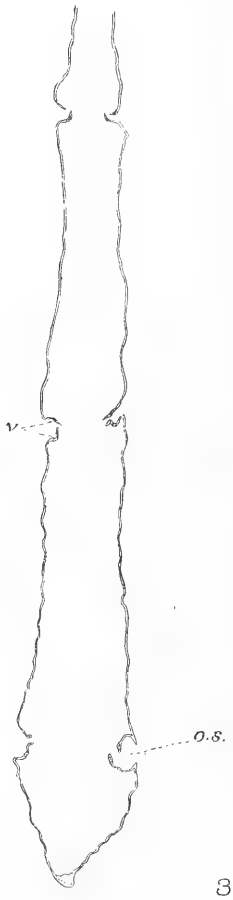

31.

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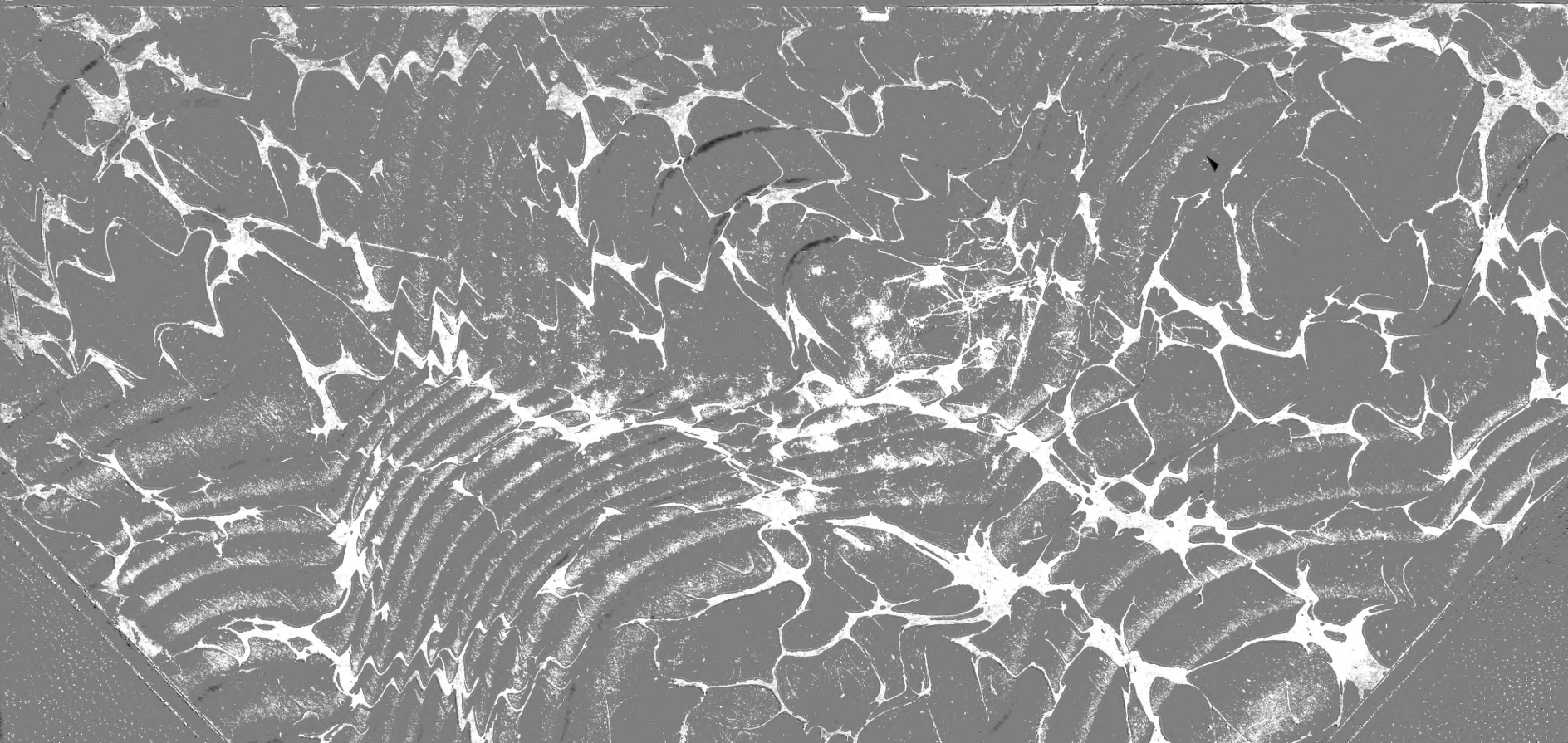
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